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A new HPLC/UV method for the determination of clindamycin in dog blood serum

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Abstract

A new HPLC method for the quantitative determination of clindamycin in dog blood serum at levels down to 80 ng/ml has been developed. Samples were deproteinised with acetonitrile and clindamycin was extracted with dichloromethane. Chromatographic analysis was carried out on a C_{18} reversed-phase analytical column in the presence of tetra-*n*-butylammonium hydrogen sulfate (TBA), as an ion-pairing agent. UV detector wavelength was set at 195 nm. The assay was validated for a concentration range from 80 to 6000 ng/ml serum. Good linearity was observed in the entire concentration range. The limit of quantification (LOQ) was 80 ng/ml and the limit of detection (LOD) was 60 ng/ml. Regression of accuracy data yielded an overall mean recovery value (±S.E.M.) of 93.98 ± 0.42%, while precision data revealed coefficient of variation (CV (%)) values lower than 4.41%. The method was successfully applied to determine drug concentrations in serum samples from dogs that had been orally administered clindamycin hydrochloride.

Keywords: Clindamycin; Dog serum; Reversed-phase HPLC

1. Introduction

Clindamycin [7(*S*)-chloro-7-deoxylincomycin] is a lincosamide antibiotic (Fig. 1). It is synthesized from microbially fermented lincomycin by replacing a hydroxyl group at the 7-position of lincomycin by a chlorine group, that significantly increases its activity. The effect of clindamycin, which is primarily bacteriostatic, is exerted by its binding to the 50S ribosomal subunit and the consequent inhibition of bacterial protein synthesis [1].

It is active against aerobic Gram-positive and anaerobic bacteria, mycoplasmas, and some protozoa. In companion animal medicine, clindamycin is mainly used in the treatment of diseases like staphylococcal skin infections and osteomyelitis, periodontal disease, bacterial prostatitis, *toxoplasmosis*, and *neosporosis* [2]. Clindamycin is launched in formulations for either oral (as clindamycin hydrochloride), or parenteral (as clindamycin 2-phosphate) administration.

Several methods have been published for the determination of clindamycin in bulk drugs and

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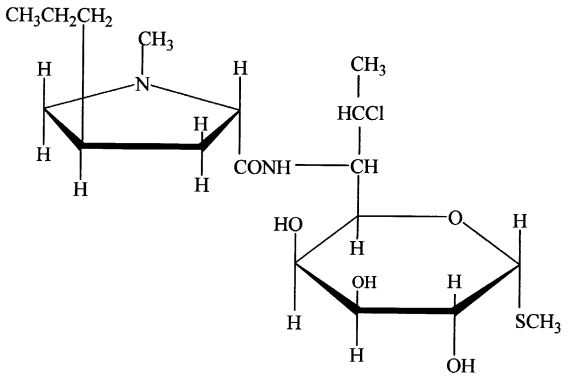

Fig. 1. Chemical structure of clindamycin.

formulations. Microbiological [3] and spectrophotometric [4] assays both suffer from lack of specificity and accuracy. Gas–liquid chromatography (GLC) [5,6] compensates for the previous, but requires relatively complicated sample manipulation. HPLC methods [7–10] are much more accurate and precise. Refractive index [7,8], electrochemical [9], and UV [8,10] detection have been applied. UV detection seems to offer more, in terms of sensitivity and stability.

Determination of clindamycin in biological samples has been performed by microbiological [11], RIA [12], GLC [13], HPLC/UV [14–16], and HPLC/MS [17–20] methods. Microbiological, gas chromatographic methods, and radioimmuno-assays are either non-specific, or time-and-reagent consuming. The HPLC/UV method developed by La Follette et al. [14] has been reported to be non-reproducible by Liu et al. [15], who presented a rather sophisticated technique, using coupled columns and two mobile phases, to extract clindamycin from human plasma samples. Fieger-Büschges et al. [16] reported a simple method involving solid-phase extraction (SPE), but the LC run was quite long (over 21 min) and the peak resolution achieved, ambiguous. HPLC/MS methods [17–20] display the highest performance; however, the LC/MS instruments are not yet readily obtainable by all laboratories working in the field of veterinary pharmacology. The method presented in this paper has been developed and validated for the purpose of a pharmacokinetic study of clindamycin in dogs, which was successfully conducted in our laboratory.

2. Experimental

2.1. Reagents

Standard clindamycin hydrochloride CRS (86.8% clindamycin base) was purchased from European Pharmacopoeia (Strasbourg, France). HPLC-grade acetonitrile and dichloromethane were from J.T. Baker (Deventer, Holland). Sodium hydroxide pellets, tetra-*n*-butylammonium hydrogen sulphate (TBA), and phosphoric acid (85% m/m) were from Merck (Darmstadt, Germany). Analytical-grade potassium di-hydrogen phosphate and di-sodium hydrogen phosphate were from Riedel-de Haën (Seelze, Germany). HPLC-grade water was produced using a Milli-Q (Millipore, Bedford, MA, USA) purification system.

2.2. Apparatus

The method was developed on a Shimadzu LC-10A series chromatographic system (Shimatzu Corporation, Kyoto, Japan). The system consisted of a Model CBM-10A controller unit, a Model DGU-2A degasser, two Model LC-10AD piston pumps, a Model SIL-10A$_{XL}$ autosampler, a Model CTO-10A column oven and a Model SPD-10AV Ultra-Violet/Visual detector. Integration was performed with the use of the Class-LC10 program (Version 1.41, Shimadzu). Separation was achieved on a MZ-Analysentechnik (Mainz, Germany) Spherisorb ODS-2 (250 mm × 4 mm i.d., 5 μm particle size), C_{18} reversed-phase analytical column.

A Model Genie-2 vortex mixer (Scientific Industries Inc., Bohemia, NY, USA), a Model Centra CL3R refrigerated centrifuge (Thermo IEC, Needman Heights, MA, USA) and a Model Reacti-Therm III evaporation unit (Pierce Chem., Rockford, IL, USA) were used for sample treatment. Mobile phase pH was determined with the use of a Model Accumet Basic pH meter (Fischer Scientific, Manchester, UK). Standard clindamycin hydrochloride was weighed on a Model AX-105 analytical balance (Mettler Toledo Inc., Greifensee, Switzerland).

2.3. Standard solutions

A stock solution of 1 mg/ml was prepared in a 10 ml volumetric flask by dissolving ca. 10 mg of

clindamycin base (ca. 11.52 mg clindamycin hydrochloride) in 10 ml HPLC-grade water. Clindamycin proved to be readily soluble in water and the stock solution was stored at 4 °C, protected from light, with the use of aluminium foil.

Successive dilutions of the stock solution in acetonitrile resulted in the preparation of four intermediate standard solutions at the following concentrations: 3, 10, 30, and 100 μg/ml. All intermediate standard solutions were stored under the same conditions as the stock solution.

Nine calibrators (working solutions) in the range of 60–8000 ng/ml (3–400 ng/50 μl injected) for clindamycin were prepared by transferring appropriate aliquots of the intermediate standard solutions in glass tubes, evaporating to dryness under a gentle stream of nitrogen (N_2) at 40 °C, and reconstituting with 500 μl of mobile phase.

2.4. Validation control (VC) samples

Validation control samples were prepared at six fortification levels (80, 180, 240, 600, 1800, and 6000 ng/ml) with a procedure similar to the one followed for the calibrators; specific aliquots of intermediate standard solutions were transferred into glass tubes. After evaporation, the dried residue was reconstituted in 0.5 ml blank dog serum and allowed to stand for 30 min. Batches of VC samples were prepared to evaluate the accuracy and the precision of the method and the stability of clindamycin in spiked serum samples.

2.5. Chromatographic conditions

The mobile phase was a mixture of acetonitrile–phosphate buffer (19:81, v/v) and contained 2.5 mM tetra-*n*-butylammonium hydrogen sulfate. The phosphate buffer was prepared by dissolving potassium di-hydrogen phosphate (20 mM) and di-sodium hydrogen phosphate (10 mM) in HPLC-grade water. The pH of the aqueous component of the mobile phase was adjusted to 3.5 with 1 M *ortho*-phosphoric acid, prior to the addition of acetonitrile. The mobile phase was filtered through a 0.2 μm, Nylon 47 mm filter (Alltech Ass. Inc., Deerfield, IL, USA), and it was degassed using helium (He).

The HPLC system operated isocratically, at a flow rate of 1.0 ml/min. Isocratic elution was carried out at 40 °C and UV detection was performed at 195 nm.

The stationary phase was thoroughly equilibrated with mobile phase each time before use. Reproducible capacity factors (k') were obtained after passage of at least 150 ml of mobile phase through the column.

2.6. Sample extraction and cleanup

Dog serum (0.5 ml) was deproteinised with the addition of acetonitrile (1 ml) followed by vortex-mixing at high speed for 1 min and centrifugation at 3500 × *g* for 5 min. The clear supernatant was transferred to a new glass tube and 50 μl of a 0.4 M sodium hydroxide solution were added. After allowing equilibrating, extraction was carried on with the addition of 6 ml of dichloromethane, vortex-mixing again at high speed for 1 min and centrifuging at 3500 × *g* for 10 min. Following centrifugation, the upper aqueous layer was discarded by aspiration, and a 5 ml aliquot of the lower, dichloromethane layer was transferred to a new glass tube and was evaporated at 40 °C, under a N_2 stream. The dried residue was reconstituted in 500 μl of mobile phase, and vortex-mixed for 15 s. After transferring the samples to autosampler inserts, 50 μl were injected in the LC system.

2.7. Determination

Calibration curves were obtained by running a total of 12 standard working solutions and plotting the recorded peak heights versus the corresponding mass of clindamycin injected, using the least-square method. Two calibration curves were used to cover the entire range of concentrations (60–8000 ng/ml): the low-concentration curve (calibrator range: 60–1200 ng/ml, or 3–60 ng/50 μl injected) for concentrations below 1000 ng/ml, and the high-concentration curve (calibrator range: 400–8000 ng/ml, or 20–400 ng/50 μl injected) for concentrations above 1000 ng/ml.

Quantification of clindamycin in an unknown sample was achieved by back-referring the displayed peak height to the appropriate corresponding calibration curve and multiplying the result of the equation $x = (y - b)/a$, where x stands for ng clindamycin/50 μl injected, y stands for peak height (in μV), a stands for curve slope and b for intercept, by

the predetermined dilution factor and overall mean recovery.

3. Results and discussion

3.1. Cleanup and extraction

The goal targeted when developing a cleanup procedure in biological matrices, like blood serum, is to achieve the maximum possible recovery, altogether with minimum interferences from endogenous compounds or reagents used to extract the investigational analyte(s). Protein precipitation with an organic solvent was preferred over use of an acid, since the latter provided less clean chromatograms, as shown in preliminary trials. Subsequent sample alkalization with 50 μl of a 0.4 M sodium hydroxide solution was performed, since a high pH is necessary to bring cationic substances (clindamycin $pK_a = 7.6$) to their non-ionized (extractable) form. Ethyl acetate and dichloromethane were chosen to be tested as organic solvents to perform the extraction with. Ethyl acetate is lighter than water, thus making the task of transferring an aliquot to a new glass tube for further procedure easier. However, interferences observed when using ethyl acetate were substantially more than when using dichloromethane. Disturbing interferences were also observed in preliminary trials not including the protein precipitation step during sample pretreatment. Therefore, a two-step liquid extraction procedure with dichloromethane under alkaline conditions was finally preferred, as recovery of clindamycin was also observed to be higher.

3.2. Chromatography

Due to the chemical characteristics of clindamycin (weak organic base), an adsorptive interaction between the substance and negatively charged silanol groups on the surface of the silica-based stationary phase was anticipated. To suppress the ionization of free, residual silanols, the use of an acidic mobile phase without the addition of an ion pairing agent, reported to cause non-reproducible retention times [19], was chosen. The tested mobile phase was a mixture of acetonitrile–10% phosphoric acid (40:60 v/v). Determination was carried out at 210 nm. A relatively good resolution of clindamycin peak from interferences was achieved, its retention time being 4.0 min. However, the eluted peak displayed a far from optimal chromatographic behavior, tailing badly, and this indicated that the undesired interaction of clindamycin with silanols had not been completely eliminated. Furthermore, the pH of the mobile phase approached the value of 2, raising questions about the stability of clindamycin and of the stationary phase.

Upon the need to use a mobile phase with a higher pH, the second effort to eliminate the aforementioned retention mechanism was based on the use of an ion pairing agent (an alkylsulfonate). Landis et al. [8] separated clindamycin from relative compounds in pharmaceuticals on a C_{18} analytical column and reported a positive correlation between ion-pair formation and the number of carbons in the alkylsulfonate molecule used as ion-pairing agent. La Follette et al. [14] also used tetramethylammonium chloride and adjusted the pH of the mobile phase to 6.7.

The use of tetra-*n*-butylammonium hydrogen sulfate was chosen, since it is an excellent choice for the determination of cationic substances with similar pK_a [21]. The pH of the aqueous phase was adjusted to 3.5, which is relatively low compared to the pH used by the majority of other investigators [8,10,14,15], although Hornedo-Nuñez et al. [9] successfully applied a mobile phase with a pH 2.5. Adjustment was performed with the use of 1 M *ortho*-phosphoric acid. Even in such pH values, silanols are expected to be substantially ionized. The addition of an ion-pairing agent in the mobile phase results in a relative “masking” of their negative charge and therefore, in a decrease in the electrostatic attraction between silanols and clindamycin. Furthermore, the theory of electrostatic repulsion of protonated analytes, like clindamycin, by positively charged tetra-*n*-butylammonium ions adsorbed onto the stationary phase has also been suggested [22].

Optimization of chromatographic conditions was achieved by varying the organic phase percentage and the amount of TBA in the mobile phase. By trial and error, an acetonitrile–phosphate buffer ratio 19:81 (v/v), and a TBA concentration of 2.5 mM gave the optimum results. New chromatographic conditions remarkably improved LC performance. The retention time was increased (clindamycin peak appeared at 5.4 min), but the peak was sharper, symmetric and

displayed excellent, baseline resolution from other, unknown identity peaks (Fig. 2B). The increase of the retention time can be attributed to the ion-pair formation between clindamycin and TBA and the relatively large size of the new molecule. Spectrum analysis between 190 and 210 nm showed the higher absorbance, along with the minimum interferences to be observed at 195 nm, without a compromise in baseline stability, reported by La Follete et al. when using wavelengths below 198 nm (Fig. 2A and C).

It can be concluded that sample pretreatment (liquid–liquid extraction) and the consequent cleanliness of samples allowed the establishment of a low limit of quantification (LOQ), since baseline noise and interferences were minimized. The high and stable recovery determined throughout the validation procedure made the use of an internal standard unnecessary.

3.3. Validation

The method was validated for use, in a 3 day validation study, with respect to linearity, selectivity, sensitivity (limit of detection (LOD) and limit of quantification), accuracy, precision, recovery, stability, and applicability aspects.

3.3.1. Calibration and linearity

The linearity of the detector response for clindamycin was evaluated by injecting six calibrators (3–60 ng/50 μl injected) for the low-concentration curve and another series of six calibrators (20–400 ng/50 μl injected) for the high-concentration curve, thus covering the entire working range of the assay. This procedure was performed five times. Least-squares linear regression and best-fit analysis of the data obtained showed the detector response for clindamycin to be linear in both curves.

The low-concentration curve was described by the following equation (y stands for peak height in μV and x for the amount of clindamycin injected (in ng/50 μl)): $y = 114.96(\pm 0.69)x + 17.12$, $r^2 = 0.999$, whereas the high-concentration curve was best described by the equation: $y = 113.70(\pm 1.15)x + 155.40$, $r^2 = 0.997$. Numbers in parentheses represent S.E.M. values, as obtained from ANOVA. P-values for intercepts were well above the significance level ($\alpha = 0.05$). Calibration curves parameters are presented in Tables 1 and 2.

3.3.2. Selectivity

The method was checked to ensure that there was no interference with clindamycin from matrix co-extractives. Chromatograms obtained from blank serum samples collected from 12 dogs showed that the peak attributable to clindamycin was resolved sufficiently from other peaks (Fig. 2) to enable reliable quantification.

3.3.3. Limit of detection and limit of quantification

The limit of detection, determined as the lowest concentration of the calibration curve, was set at 60 ng/ml.

Table 1
Low-concentration calibration curve parameters of clindamycin

Curve	Peak height (μV)						Slope	y-Intercept	r^2
	3[a]	6[a]	12[a]	20[a]	40[a]	60[a]			
1	360	704	1356	2261	4499	6659	110.72	37.99	0.9999
2	389	671	1377	2297	4748	6913	115.97	7.29	0.9995
3	354	710	1397	2326	4706	6996	116.79	3.59	0.9999
4	359	729	1388	2391	4611	7072	117.05	7.61	0.9997
5	352	706	1375	2364	4670	6829	114.18	32.74	0.9996
Mean	362.80	704.00	1378.60	2327.80	4646.80	6893.80	114.96	17.12	
S.D.	15.02	20.94	15.44	51.77	96.71	159.61			
S.E.M.							0.68	21.32	
CV (%)	4.14	2.97	1.12	2.22	2.08	2.32			

[a] Clindamycin injected (ng/50 μl).

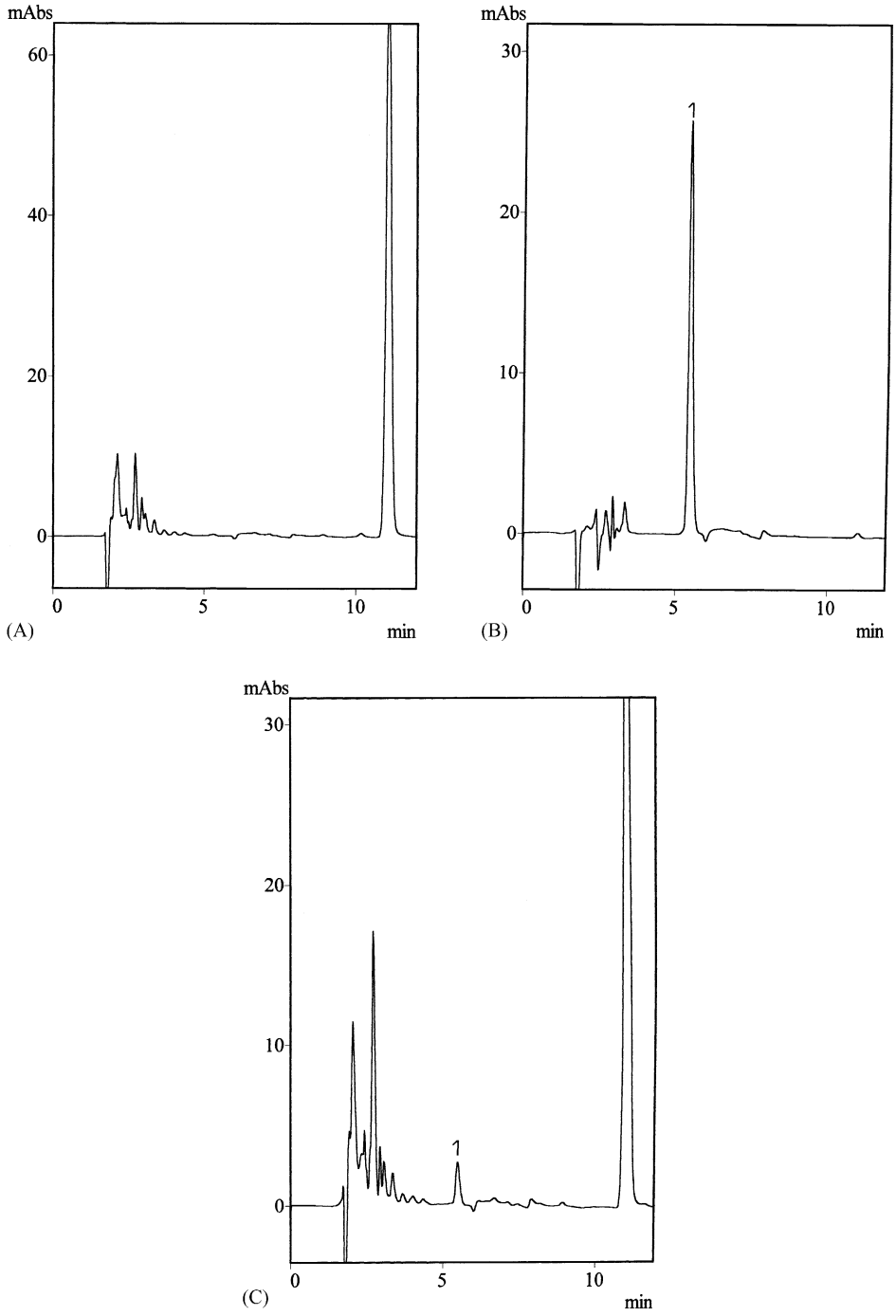

Fig. 2. (A) Typical chromatograms of a blank dog serum sample; (B) of a standard clindamycin (peak 1) solution (4000 ng/ml) in mobile phase; and (C) of a sample spiked with 600 ng/ml clindamycin. Stationary phase: Spherisorb ODS-2; mobile phase: acetonitrile–phosphate buffer (19:81, v/v), containing 2.5 mM tetra-*n*-butylammonium hydrogen sulfate; column temperature: 40 °C; flow-rate: 1 ml/min; wavelength, 195 nm; injection volume: 50 μl.

Table 2
High-concentration calibration curve parameters of clindamycin

Curve	Peak height (μV)						Slope	*y*-Intercept	r^2
	20[a]	40[a]	60[a]	120[a]	200[a]	400[a]			
1	2330	4572	6659	13,570	22,252	43,054	107.307	383.18	0.9997
2	2287	4648	6913	13,864	23,917	48,238	121.165	−318.66	0.9999
3	2326	4656	6996	14,567	23,871	45,119	112.964	440.95	0.9988
4	2351	4611	7072	13,949	23,931	45,403	113.796	288.10	0.9992
5	2364	4670	6829	13,585	22,044	45,559	113.274	−16.56	0.9996
Mean	2331.60	4631.40	6893.80	13,907.0	23,203.0	45,474.6	113.70	155.40	
S.D.	29.36	39.73	159.61	405.11	966.14	1,846.24			
S.E.M.							1.148	219.89	
CV (%)	1.26	0.86	2.32	2.91	4.16	4.06			

[a] Clindamycin injected (ng/50 μl).

Table 3
Accuracy data of the analysis of dog serum spiked with clindamycin

Clindamycin added (ng/ml)	Overall mean concentration found[a] (ng/ml ± S.D.)	Mean recovery (% ± S.D.)	Overall recovery (% ± S.E.M.)
80	80.70 ± 2.85	100.88 ± 3.56	
240	242.39 ± 3.92	100.99 ± 1.64	
600	570.54 ± 21.21	95.09 ± 3.53	93.98 ± 0.42
1800	1697.66 ± 52.21	94.31 ± 2.90	
6000	5648.37 ± 224.26	94.14 ± 3.74	

[a] Twenty four replicates.

The limit of quantification was defined as the lowest fortification level (80 ng/ml) validated, in terms of accuracy and precision. Accepted criteria demand percent bias and coefficient of variation (CV (%)) values less than 20%. During the validation phase, 24 replicates ($n = 24$) were analyzed, and accuracy (bias = 0.88%) and precision (CV = 3.53%) data permitted the establishment of the method LOQ at 80 ng/ml.

3.3.4. Accuracy and precision

The accuracy of the method, in terms of recovery efficiency, is a measure of the response of the analytical method to the entire quantity of the analyte contained in a sample. The recovery was studied by fortifying blank serum samples with clindamycin at five fortification levels (80, 240, 600, 1800, and 6000 ng/ml) and analyzing 24 ($n = 24$) replicates. The recover-

Table 4
Precision data of the analysis of dog serum spiked with clindamycin, on three successive days

Clindamycin added (ng/ml)	Mean concentration found[a] (ng/ml ± S.D.)			CV (%)		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
80	80.14 ± 2.73	80.65 ± 3.50	81.31 ± 2.49	3.41	4.34	3.07
240	242.36 ± 2.22	242.31 ± 4.80	242.39 ± 4.75	0.92	1.98	1.96
600	573.78 ± 19.61	565.51 ± 20.30	572.33 ± 25.23	3.42	3.59	4.41
1800	1704.89 ± 53.89	1674.43 ± 56.01	1713.65 ± 44.13	3.16	3.34	2.58
6000	5636.36 ± 200.6	5509.11 ± 241.4	5799.63 ± 135.1	3.56	4.38	2.33

[a] Eight replicates.

Table 5
Stability of clindamycin in spiked serum samples after 24 h waiting time in the autosampler (room temperature, approximately 22 °C), after 4 months storage at −30 °C and after a three freeze-thaw cycle

Clindamycin added (ng/ml)	Mean concentration found[a] (ng/ml ± S.D.)		*P*-value[b] (two-tail paired *t*-test)
	0 h	24 h	
Autosampler			
80	81.39 ± 2.17	80.57 ± 0.95	0.379
600	594.65 ± 15.00	570.90 ± 13.85	0.788
1800	1686.20 ± 21.24	1688.10 ± 28.87	0.834
	0 months	4 months	
Storage at −30 °C			
180	178.87 ± 1.47	179.24 ± 1.25	0.477
600	556.63 ± 14.42	555.06 ± 13.36	0.385
1800	1626.24 ± 82.79	1618.90 ± 77.61	0.669
	Before	After	
Three freeze-thaw cycle			
600	572.52 ± 25.59	569.09 ± 30.52	0.896
1800	1605.35 ± 21.26	1598.70 ± 17.86	0.756

[a] Six replicates.
[b] Significance level, $\alpha = 0.05$.

ies determined ranged from 94.14 to 100.99%. The overall mean recovery was determined after plotting the determined ("found") versus the actual ("added") concentrations. Least-square linear regression analysis showed that the relationship between "added" (x, in ng/ml) and "found" (y, in ng/ml) could be described by the equation: $y = 0.9398(\pm 0.004)x + 8.99$. The number in parenthesis represents the SEM and the *P*-value for the intercept was 0.45 (>0.05). The slope of the curve could be therefore used as an estimate of the overall mean recovery of clindamycin (93.98 ± 0.4%).

The precision of the method was evaluated utilizing samples fortified at the previous five levels, which were analyzed on three successive days. The coefficients of variation (CV (%)) of the concentrations found were calculated. The results showed CV values ranging from 0.92 to 4.41%. Data concerning the accuracy and precision of the method are presented in Tables 3 and 4.

3.3.5. Storage stability

Data on clindamycin stability in fortified serum samples are summarized in Table 5. To evaluate clindamycin stability in samples pending analysis in the autosampler, a fundamental prerequisite for a reliable daily analysis, drug-free serum samples were spiked

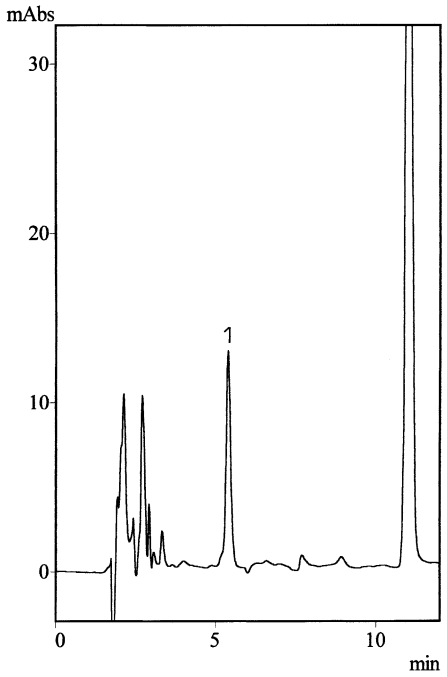

Fig. 3. A typical chromatogram of serum sample from a dog orally administered a single dose of 11 mg/kg b.w. clindamycin (sample collected 1 h after drug administration; clindamycin concentration: 139.84 ng/50 μl injected). Chromatographic conditions and peak identification as in Fig. 2.

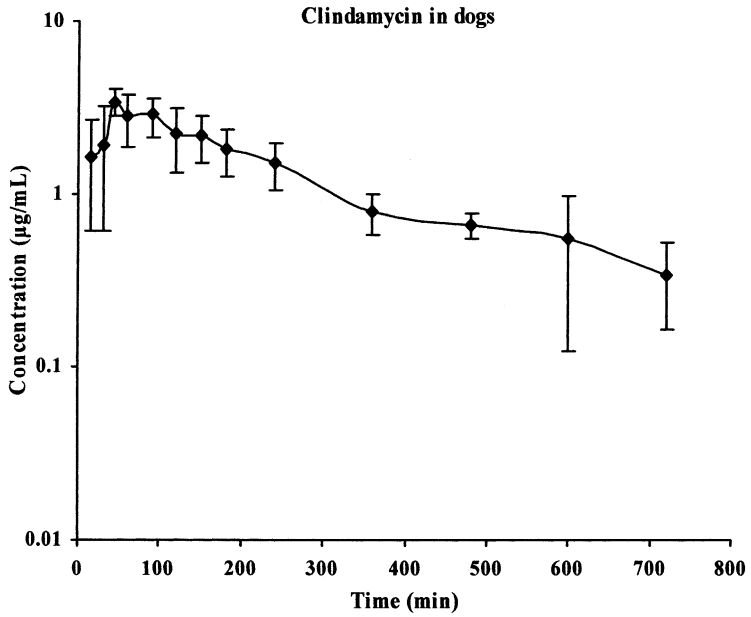

Fig. 4. Clindamycin concentration–time profile in dog serum. Samples were collected from four dogs.

at 80, 600, and 1800 ng/ml. After extraction, samples were arranged in the autosampler and were analyzed immediately and after 24 h. Statistical analysis of the results (paired *t*-test) did not reveal the average of the differences between the two measurements to be significantly different from zero.

After assessment of clindamycin stability throughout daily analysis, two more stability experiments were conducted. For the first experiment, blank samples were spiked at 180, 600, and 1800 ng/ml and were analyzed before and after 4 months storage at −30 °C. For the second experiment, blank samples fortified at 600 and 1800 ng/ml underwent a three freeze-thaw cycle. In all cases, statistical analysis of the data (paired *t*-test) confirmed that sample storage under the investigational conditions had no significant effect on clindamycin stability.

3.3.6. Applicability

The present method proved to be a reliable tool for the quantitative determination of clindamycin in dog blood serum, as it has been successfully applied in a pharmacokinetic study of clindamycin after per os administration of clindamycin hydrochloride (Antirobe® capsules, 150 mg) to four ($n = 4$) dogs of the Beagle breed (average age: 18 months), at the nominal dose of 11 mg/kg b.w. Blood samples were collected prior to ($t = 0$ h) and at 15, 30, 45, 60, 90, 120, 150, 180, 240, 360, 480, 600, 720, and 1440 min after drug administration. Blood samples were allowed to form a clot at ambient temperature (approximately 22 °C) and, following centrifugation at 1500 × *g* for 15 min, the supernatant serum was stored at −30 °C, pending analysis with the specified method. A typical chromatogram of a serum sample collected from a dog that had been orally administered a single dose of clindamycin hydrochloride is illustrated in Fig. 3 and the concentration–time profile of clindamycin in serum (obtained from all animals) is illustrated in Fig. 4.

4. Conclusion

The HPLC method described in this paper offers economy (in terms of low cost of apparatus chemicals and reagents required) and satisfactory sample throughput (roughly 80 samples in a total time of 5 h, by a single analyst), selectivity, sensitivity and chromatographic characteristics. This method, applicable to real samples, can be considered suitable and become

incorporated in routine analysis for the determination of clindamycin in blood serum.

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