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DETERMINATION OF CLINDAMYCIN IN PHARMACEUTICALS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING ION-PAIR FORMATION

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SUMMARY

A reversed-phase ion-pairing high-performance liquid chromatographic procedure with refractive index or UV 214 nm detection was developed for the separation of clindamycin, clindamycin B, and 7-epiclindamycin. The chromatographic retention behavior of these compounds on an octadecylsilane column was investigated as a function of pairing-ion, mobile phase composition, and pH. The method was applied to the determination of clindamycin in bulk drug and in a number of pharmaceutical formulations. The relative standard deviations for all assays was in the 0.5–2% range.

INTRODUCTION

Clindamycin (V, Fig. 1) is a highly effective antibiotic against Gram positive aerobes and both Gram negative and Gram positive anaerobic pathogens[1]. It is synthesized from Lincomycin (I, Fig. 1), an antibiotic produced by microbial fermentation, by substituting chlorine for the hydroxyl group in the 7 position which results in an increase in biological activity.
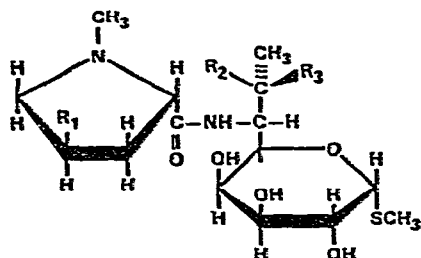
I	LINCOMYCIN	$R_1 = CH_3-CH_2-CH_2-$	$R_2 = OH$	$R_3 = H$
II	LINCOMYCIN B	$R_1 = CH_3-CH_2-$	$R_2 = OH$	$R_3 = H$
III	CLINDAMYCIN B	$R_1 = CH_3-CH_2$	$R_2 = H$	$R_3 = Cl$
IV	7-EPICLINDAMYCIN	$R_1 = CH_3-CH_2-CH_2-$	$R_2 = Cl$	$R_3 = H$
V	CLINDAMYCIN	$R_1 = CH_3-CH_2-CH_2-$	$R_2 = H$	$R_3 = Cl$

Fig. 1. Structures of clindamycin and related compounds.

Common impurities in clindamycin bulk drug are clindamycin B (III), 7-epi-clindamycin (IV), and a small amount of the lincomycin starting material (Fig. 1). Clindamycin B is formed from the small amount of lincomycin B (II) which is a normal by-product produced during fermentation; 7-epiclindamycin is produced during the synthesis of clindamycin, presumably by attack of chloride ion under acid conditions on an oxazoline intermediate[2].

Several methods have been reported for the determination of clindamycin in bulk drug and dosage forms including a microbiological method[3], gas–liquid chromatography (GLC)[4], and high-performance liquid chromatography (HPLC)[5]. The microbiological method is time-consuming and only measures total activity. The GLC method requires an extraction and a derivatization step. The HPLC method, although rapid, does not separate the epimers, clindamycin and 7-epiclindamycin.

The present study was undertaken to develop a rapid HPLC method capable of separating lincomycin, clindamycin, 7-epiclindamycin, and clindamycin B. The effects of mobile phase composition, pH, and pairing-ion were studied to find conditions where all compounds are separated.

EXPERIMENTAL

Apparatus

A modular HPLC was used for this work consisting of a single-piston pulse-dampened Altex pump (Model 110A, Berkeley, CA, U.S.A.); a syringe-loaded six-port injection valve fitted with a 10- or a 25-μl loop (Model 7120, Rheodyne, Berkeley, CA, U.S.A.); a 30 cm × 3.9 mm I.D. μBondapak® C_{18} column (No. 27324, Waters Assoc., Milford, MA, U.S.A.); a differential refractometer detector (Model R401, Waters Assoc.); a 214-nm fixed-wavelength UV detector with a Zn-line source (Model 1203, Laboratory Data Control, Riviera Beach, FL, U.S.A.); and a 10-mV recorder operated at 0.5 cm min^{-1} (Model XKR, Sargent Welch, Skokie, IL, U.S.A.). Data were collected and processed using the computer system developed in house[6]. pH values were measured on the final methanol–water mobile phase so only "apparent" values are measured.

Reagents

Sterile, double-distilled water; acetic acid (No. 2504, Mallinckrodt, St. Louis, MO, U.S.A.); distilled-in-glass UV grade methanol (Burdick & Jackson, Muskegon, MI, U.S.A.); and internal standard phenethyl alcohol (No. 313, Eastman, Rochester, NY, U.S.A.) were used as received. All the ion-pairing reagents were from Eastman: ethane sulfonic acid (No. 6217), sodium butane sulfonate (S035), sodium pentane sulfonate (No. 10178), sodium hexane sulfonate (No. 10263), sodium octane sulfonate (No. 10265), and D,L-10-camphor sulfonate (No. 5057).

Samples of clindamycin B, 7-epiclindamycin, clindamycin, lincomycin, and lincomycin B all as hydrochloride salts were supplied by the Fine Chemical Division of The Upjohn Company. The purity of the clindamycin reference standard was determined by the Control Division of The Upjohn Company using several complementary techniques.

Mobile phase

All mobile phases contained 0.005 M pairing-ion in methanol–water mixtures. After mixing, the mobile phases were adjusted to the desired pH with 1 M sodium hydroxide or hydrochloric acid, filtered through a 5-μm filter, and degassed by sonication and vacuum.

Two mobile phases were used for analysis of samples. When the differential refractometer was used as the detector, the mobile phase was composed of methanol–water (60:40), 2 ml glacial acetic acid per liter (0.035 M), and 0.005 M D,L-10-sodium camphor sulfonate, adjusted to pH 6.0. When 214 nm UV detection was used, the mobile phase was composed of methanol–water (60:40), 0.01 M phosphate buffer, and 0.005 M sodium pentane sulfonate.

The column was operated at 900 p.s.i.g. at a flow-rate of 1.0 ml min^{-1} for all experiments.

Sample preparation and analysis

Bulk drug samples were prepared by dissolving accurately weighed samples (ca. 15 mg) in 1.0 ml of internal standard solution (0.5 ml of phenethyl alcohol in 100 ml of mobile phase). Aliquots (25 μl) of the samples were chromatographed, corresponding to 375 μg on column, at a detector setting of 16 $\times$, 3.8·10^{-4} refractive index units full scale, when the differential refractometer was used as a detector. Amounts of clindamycin were determined by comparison of peak height or peak area ratios from the sample preparation to those obtained from a standard prepared in an analogous manner.

Hard-filled capsules (HFCs) were prepared by emptying the contents of 10 capsules, mixing, weighing an amount equivalent to ca. 75 mg of clindamycin base, extracting for 30 min with mobile phase containing internal stadard, filtering, and chromatographing.

Syrups were prepared by pipetting an amount equivalent to 50 mg of clindamycin base, adding 5 ml of internal standard, and chromatographing.

When added sensitivity is required, a 214 nm UV detector is employed using a mobile phase containing pentane sulfonate. In this case, the amount of clindamycin injected on column can be reduced by a factor of four to obtain an equivalent signal-to-noise ratio with the differential refractometer detector.

RESULTS AND DISCUSSION

The most difficult compounds to separate in developing this procedure were the epimers, clindamycin (V) and 7-epiclindamycin (IV). They differ structurally only in the configuration of the chlorine and hydrogen in the 7 position. Since clindamycin is extremely water-soluble (800 g l^{-1}) and is protonated at the normal operating range of most HPLC columns (pK_a 7.6), a reversed-phase ion-pairing system was used.

Chromatography was optimized by varying the methanol–water ratio, pH, and the ion-pairing reagent in the mobile phase.

A methanol–water ratio of 60:40 (v/v) gave a capacity factor for clindamycin in the 1–4 range. Under these conditions, clindamycin and 7-epiclindamycin coeluted. Addition of D,L-10-camphor sulfonate to the mobile phase sharpened the peaks and greatly reduced tailing by ion-pairing with the charged tertiary amine.

The greatest improvement in the selectivity of the mobile phase was obtained by varying the pH. The dependence of the capacity factors on the mobile phase pH in the range 2.5–6.5 for compounds I–V are given in Fig. 2. Lincomycin and lincomycin B eluted close to the solvent front independent of the mobile phase pH, demonstrating the importance of the substituted propyl group on retention. Clindamycin and 7-epiclindamycin are more non-polar than clindamycin B, propyl group vs. ethyl group on the pyrrolidine ring, and elute later at all pH values. At low pH values, clindamycin and 7-epiclindamycin coelute. In the pH range 5–6.2, clindamycin and 7-epiclindamycin are baseline resolved. With further increase in basicity, the retention of clindamycin becomes longer and 7-epiclindamycin and clindamycin B coelute.
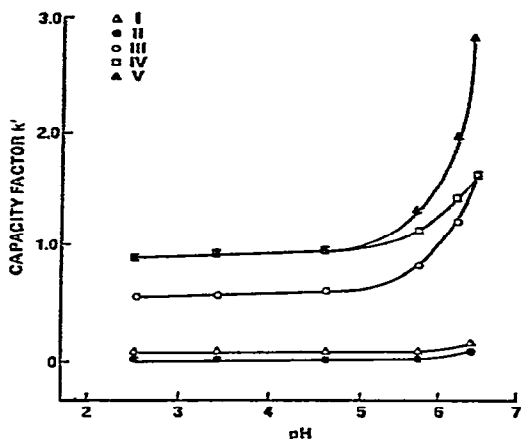

Fig. 2. Dependence of the capacity factors (k') of compounds I–V on the apparent pH of the mobile phase. Chromatography was performed on a 10-μm μ-Bondapak C_{18} column with a mobile phase of methanol–water (60:40), 0.01 M ammonium nitrate, 0.005 M camphor sulfonate, and 0.035 M acetic acid. The pH was adjusted with 0.1 M HCl or NaOH.

The effect of chain length of the pairing-ion on the capacity factors of compounds I–V was studied. Fig. 3 shows a plot of capacity factor vs. the length of straight-chain alkyl sulfonates C_2–C_8. The length of the chain had no effect on the retention of lincomycins and little effect on the clindamycins with pairing ions less than five carbons. Capacity factors markedly increased with pairing ions above five carbons. The greatest resolution between clindamycin B, 7-epiclindamycin and clindamycin was with five- and six-carbon pairing ions. Compounds I–V had similar retention time with camphor sulfonate and pentane sulfonate.

Typical chromatography of a synthetic mixture of compounds I–V is given in Fig. 4 and the capacity factors and retention times are given in Table I. Under the optimum conditions of the method, the clindamycins were baseline resolved and the lincomycins coeluted near the solvent front. Use of more polar columns, cyano or phenyl, and less polar mobile phases failed to increase the retention of the polar lincomycins.
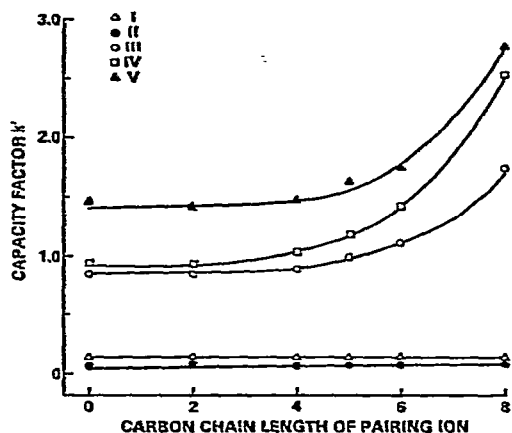
Fig. 3. Dependence of the capacity factors (k') of compounds I–V on the carbon chain length of the pairing ion. All pairing ions were 0.005 M straight-chain alkyl sulfonates. Mobile phase pH 6, all other conditions as given in Fig. 2.
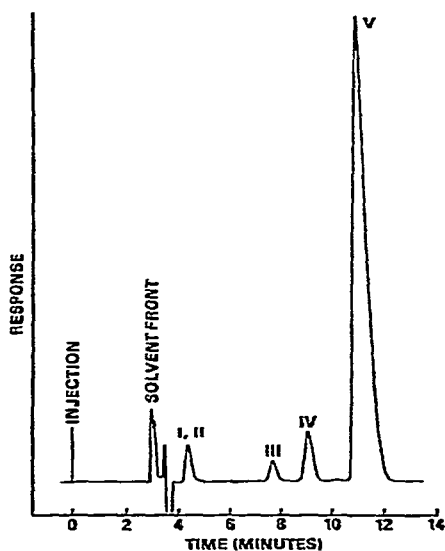

Fig. 4. HPLC chromatogram of a synthetic mixture of compounds I–V. Chromatography was performed on a 10-μm μBondapak C_{18} column with a mobile phase of methanol–water (60:40), 0.035 M acetic acid, and 0.005 M camphor sulfonate at pH 6. The flow-rate was 1.0 ml min^{-1} at 900 p.s.i.g. Compounds were detected with a differential refractometer calibrated to $3.8 \cdot 10^{-4}$ refractive index units full scale.

The chromatographic system developed is useful for quality control and stability testing and has been applied to the determination of clindamycin in pharmaceutical formulations, HFCs, and syrup.

A series of HFC and syrup placebos were spiked with amounts of clindamycin reference standard from 50–150% of label to demonstrate the recovery and precision

TABLE I

CHROMATOGRAPHIC CHARACTERISTICS OF CLINDAMYCIN, POTENTIAL IMPURI-
TIES, OR DEGRADATION PRODUCTS

Chromatography conditions are given in Fig. 4.

Compound	Retention volume (ml)*	Relative retention volume	Capacity factor
Lincomycin B	4.41	0.39	0.12
Lincomycin	4.50	0.40	0.14
Phenethyl alcohol (internal standard)	5.90	0.53	0.49
Clindamycin B	7.69	0.68	0.95
7-Epiclindamycin	9.15	0.81	1.31
Clindamycin	11.3	1.00	1.86

  * The column void volume was 3.95 ml.

TABLE II

DATA FOR THE ANALYSIS OF CLINDAMYCIN HYDROCHLORIDE HARD-FILLED
CAPSULES*

(a) Recovery data (mg/HFC)

Amount added (mg)	Amount found (mg)	Recovery (%)
25.1	25.5	101.0
50.4	49.4	98.0
75.4	76.2	101.0
100.5	100.4	99.9
150.5	150.1	99.7
220.3	219.6	99.7
		Average: 99.9%
		Rel. S.D.: 1.10%

(b) Precision data

Theory 25 mg/HFC	Theory 75 mg/HFC	Theory 150 mg/HFC
24.9	77.7	150.0
25.1	76.1	149.0
25.2	74.6	147.0
24.8	75.2	152.0
24.9	75.1	151.0
25.0	75.0	153.0
Average: 25.0	Average: 75.6	Average: 150.0
Rel. S.D.: 0.60%	Rel. S.D.: 1.50%	Rel. S.D.: 1.43%

    * Placebo tablets contain clindamycin 25–150 mg; lactose, corn starch, talc, and magnesium
stearate.

of the procedure. Recovery was quantitative by peak height and by peak area responses
for both formulations (Tables IIa and IIIa). Precision was 1.5% and 2.6% relative
standard deviation for the HFC and syrup formulations, respectively (Tables IIb and
IIIb). A typical chromatogram of the HFC formulation spiked with clindamycin B
and 7-epiclindamycin is given in Fig. 5. Total analysis time is *ca.* 13 min. Chromato-
graphy for the syrup formulation was similar. Data for the analysis of typical produc-
tion lots of HFC and syrup are given in Table IV.

TABLE III

DATA FOR THE ANALYSIS OF CLINDAMYCIN HYDROCHLORIDE SYRUP

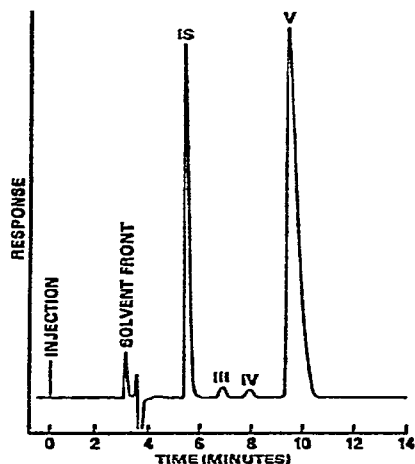| (a) Recovery data (mg/ml syrup)			(b) Precision data (theory 25 mg/ml)
Amount added (mg)	Amount found (mg)	Recovery (%)	
34.3	34.9	101.7	26.3
44.4	45.2	101.8	25.1
54.5	55.1	101.1	26.2
60.5	61.2	101.2	26.2
71.2	70.2	98.6	25.0
86.0	86.0	100.0	24.9
		Average: 100.7%	24.9
		Rel. S.D.: 1.23%	Average: 25.4
			Rel. S.D.: 2.60%

Fig. 5. HPLC chromatogram of a typical lot of 75 mg HFC spiked with clindamycin B and 7-epiclindamycin containing internal standard, phenethyl alcohol. Conditions as given in Fig. 4.

TABLE IV

ANALYSES OF CLINDAMYCIN HFCs AND SYRUP

Sample	Theory (mg/dose)	Found (mg/dose)
HFC	25	25.3
HFC	25	26.1
HFC	75	75.6
HFC	75	74.2
HFC	150	150.0
HFC	150	149.0
Syrup	25	24.9
Syrup	25	25.2

This system was also used for the analysis of bulk drug samples. Here, the amount of clindamycin B and epiclindamycin was quantitated by peak area response. The response factors of clindamycin, clindamycin B, and 7-epiclindamycin were equal within experimental error using refractive index and UV 214 nm detection, so the percentage of impurity present could be calculated by summing the area of all peaks and calculating the amount by peak area per cent. The detection limit for clindamycin B and 7-epiclindamycin was *ca.* 0.2% using this method. Clindamycin was quantitated by comparison to a reference standard. The results of the analysis of bulk drug are given in Table V showing excellent recovery and precision.

TABLE V

DATA FOR THE ANALYSIS OF CLINDAMYCIN HYDROCHLORIDE BULK DRUG

Amount added (mg)	Amount found (mg)	Recovery (%)
4.91	4.92	100.2
9.86	9.88	100.2
13.25	13.15	99.2
22.18	22.2	100.0
32.17	32.3	100.4
44.26	43.98	99.4
		Average: 99.9
		Rel. S.D.: 0.49%

If additional sensitivity is required, the camphor sulfonate can be replaced by pentane sulfonate and UV 214 nm detection can be employed.

REFERENCES

1 A. A. Forist, R. M. DeHaan and C. M. Metzler, *J. Pharmacokinet. Biopharm.*, 1 (1973) 89.
2 E. J. Hessler and R. G. Robinson, Upjohn, Kalamazoo, MI, personal communication, 1979.
3 *Code of Federal Regulations*, Title 21, Part 453,658 (April 1, 1977).
4 L. W. Brown, *J. Pharm. Sci.*, 63 (1974) 1597.
5 L. W. Brown, *J. Pharm. Sci.*, 67 (1978) 1254.
6 L. E. Fox, R. A. Johnson and N. D. Young, *Ind. Res. Develop.*, 20 (1978) 64.