GLC Determination of Clindamycin and Related Compounds

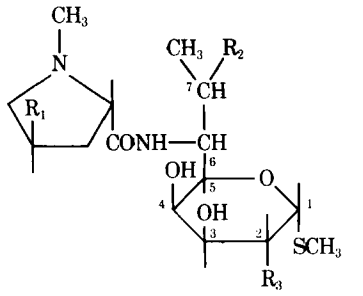
LEO W. BROWN

Abstract □ GLC assays were developed for clindamycin hydrochloride, clindamycin 2-phosphate, clindamycin 2-palmitate hydrochloride, and formulations containing these compounds. Procedures for the determination of possible related trace contaminants were also developed for evaluating the purity of these antibiotics. Different derivatives and GLC behavior of these compounds are discussed.

Keyphrases □ Clindamycin and related compounds—GLC determination of trace contaminants and impurities □ GLC—analysis, clindamycin and related compounds, determination of trace contaminants and impurities

Clindamycin (I), an antibiotic synthesized from lincomycin (II), is produced by bacterial fermentation. It has been assayed and characterized by degradation pathways by GLC (1, 2). Clindamycin 2-phosphate[1] (III) and clindamycin 2-palmitate[1] (IV) are synthesized from clindamycin. The former was developed for use in sterile injection preparations, eliciting a relatively pain-free response at the injection site in comparison with clindamycin hydrochloride. The latter is a microbiologically inactive palmitate ester of clindamycin and is readily cleaved *in vivo* to the active clindamycin. It is devoid of the characteristic bitter taste of clindamycin hydrochloride, making it suitable for oral pediatric formulations (3).

The present study was aimed at developing methods to characterize and quantitate clindamycin hydrochloride, clindamycin 2-phosphate, and clindamycin 2-palmitate hydrochloride in bulk quantities and formulations. This study involved detection of possible trace contaminants such as lincomycin, epilincomycin (V), epiclindamycin (VI), and clindamycin B (VII) in clindamycin hydrochloride. The same trace impurities would be present as the corresponding esters in clindamycin 2-phosphate and clindamycin 2-palmitate hydrochloride, since these two antibiotics are synthesized from bulk clindamycin hydrochloride. Assays of clindamycin hydrochloride and clindamycin 2-palmitate hydrochloride involved derivatization of the compounds followed by GLC. Since derivatized clindamycin 2-phosphate could not be chromatographed satisfactorily, it was enzymatically hydrolyzed to clindamycin prior to derivatization and GLC.



	R_1	R_2	R_3
I:	*n*-propyl	Cl (*S*)	OH
II:	*n*-propyl	OH (*R*)	OH
III:	*n*-propyl	Cl (*S*)	phosphate
IV:	*n*-propyl	Cl (*S*)	palmitate
V:	*n*-propyl	OH (*S*)	OH
VI:	*n*-propyl	Cl (*R*)	OH
VII:	ethyl	Cl (*S*)	OH

EXPERIMENTAL

Clindamycin Hydrochloride—*GLC Conditions*—A gas chromatograph[2] equipped with a flame-ionization detector was used. The U-shaped column was glass, 3 mm i.d. × 61 cm, packed with 3% OV-17 on 60–80-mesh Gas Chrom Q. The following temperatures were maintained: column, 170°; detector, 200°; and flash heater, 170°. The carrier gas (helium) was maintained at a flow of 60 ml/min at the operating temperature. The sample volume injected was 1 μl, with the attenuation adjusted to give at least 50% of full scale on the recorder. The sample was injected directly into the glass column.

Internal Standard Solution—A chloroform solution containing approximately 2 mg of hexacosane/ml was prepared.

Reference Standard and Bulk Drug Preparation—Accurately weighed bulk drug and reference standard samples of approximately 5 mg were placed in 15-ml glass-stoppered centrifuge tubes, 2.0 ml of the internal standard solution was added, and the solutions were then treated as described under *Procedure*.

Reference Standard and Hard-Filled Capsules Preparation—The average capsule fill weight was determined, and a sample equivalent to about 13 mg of clindamycin hydrochloride was accurately weighed into a 15-ml stoppered centrifuge tube. A reference standard clindamycin hydrochloride sample was prepared in the same manner. To the centrifuge tubes were added 6.0 ml of the internal standard solution and 6.0 ml of 1% sodium carbonate solution. The tubes were shaken vigorously and centrifuged. Then the top aqueous layers were discarded and approximately 2 g of anhydrous sodium sulfate was added to dry the chloroform layers. Two-milliliter aliquots of the chloroform solutions were placed into 15-ml glass-stoppered centrifuge tubes and treated as described under *Procedure*.

Procedure—To the reference and sample tubes was added 0.5 ml of trifluoroacetic anhydride. After swirling to mix thoroughly, the tubes were stoppered and placed in a heating block or oil bath to an approximate depth of 5 cm so that the upper part of the centrifuge tube acted as a reflux condenser. The tubes were heated at 45° for 30 min. On removal from the heating block, approximately 1 mg of anhydrous sodium carbonate was added to each tube and the tubes were allowed to stand for approximately 30 min before chromatography.

Clindamycin 2-Phosphate—*GLC Conditions*—The conditions were the same as for clindamycin hydrochloride.

Internal Standard Solution—A chloroform solution containing approximately 0.45 mg of hexacosane/ml was prepared.

pH 9 Borate Buffer with Magnesium—To a 1-liter volumetric

[1] The USAN generic name clindamycin phosphate refers to the 2-phosphate, and the generic name clindamycin palmitate refers to the 2-palmitate.

[2] F & M 402.

Table I—Impurity Results of Seven Replicate Samples of a Research Lot of Clindamycin Hydrochloride

Impurity	Average Percent	Coefficient of Variation
Epilincomycin	2.30	5.80
Lincomycin	0.225	17.2
Clindamycin B	2.20	1.95
Epiclindamycin	0.656	12.8

flask were added 3.1 g of boric acid and 500 ml of water. After mixing, 21 ml of 1 *N* sodium hydroxide and 10 ml of 0.1 *M* magnesium chloride were added. The solution was diluted to volume with water and shaken.

Reference Standard Solution—Approximately 9.5 mg of clindamycin hydrochloride hydrate was accurately weighed and placed in a 35-ml stoppered centrifuge tube; then 20 ml of pH 9 borate buffer was added.

Sample Preparation—A sterile solution of clindamycin 2-phosphate (150 mg clindamycin base equivalents/ml) was diluted with borate buffer to produce a solution containing approximately 0.4 mg of clindamycin base/ml. Twenty-five milliliters of this solution was added to a 50-ml stoppered centrifuge tube, 10 ml of chloroform was added, and the tube was shaken vigorously for 15 min. Then the tube was centrifuged and a 20-ml portion of the aqueous solution was removed with a pipet and placed in a 35-ml centrifuge tube (chloroform layer was used for determination of free clindamycin).

Procedure—To the 35-ml tubes was added 50 units of intestinal alkaline phosphatase[3]. The tubes were allowed to stand until the phosphatase dissolved and then were placed in a 37° water bath for 2.5 hr. After the tubes were removed from the water bath and cooled, 10 ml of the internal standard solution was added to each tube. The tubes were shaken vigorously for 15 min and centrifuged. Then the clear aqueous layer was discarded and the tubes were centrifuged again if an emulsion layer was present.

The remaining aqueous layer was removed by suction, and 3.0 ml of the chloroform layers was transferred to 4-ml tablet vials containing approximately 1 g of anhydrous sodium sulfate. After shaking the vials to dry the chloroform solutions, 1.0-ml aliquots of the solutions were transferred to 15-ml stoppered centrifuge tubes and 0.25 ml of trifluoroacetic anhydride was added to each. They were placed in a 45° heating block for 30 min. On removal from the heating block, approximately 1 mg of anhydrous sodium carbonate granules was added to each tube and the solutions were chromatographed.

Clindamycin 2-Palmitate Hydrochloride—*GLC Conditions*—The same conditions as for clindamycin hydrochloride were used, except the column was packed with 1% SE-54 on 60–80-mesh Diatoport S. The following temperatures were maintained: column, 260°; detector, 285°; and flash heater, 260°.

Internal Standard Solution—A chloroform solution was prepared containing approximately 5 mg of cholesteryl benzoate/ml.

Reference and Bulk Drug Preparation—Approximately 20 mg each of reference and bulk drug clindamycin 2-palmitate hydrochloride was accurately weighed and transferred to 15-ml stoppered centrifuge tubes, and 1 ml of the internal standard solution was added.

Flavored Granules Sample Preparation—A flavored granules sample, containing approximately 80 mg of clindamycin 2-palmitate hydrochloride, was accurately weighed into a 15-ml centrifuge tube. Four milliliters of the internal standard solution was added, and the mixture was shaken vigorously and centrifuged. A 1.0-ml aliquot of the clear solution was transferred to another 15-ml centrifuge tube.

Procedure—One milliliter of pyridine and 0.5 ml of acetic anhydride were added and the tubes (reference and sample) were gently swirled for complete mixing. They were stoppered and placed in a heating block as in the procedure for clindamycin hydrochloride, except that the heating was at 100° for 2.5 hr. The solutions were removed from the block, allowed to cool, and chromatographed.

[3] Worthington Biochemical Corp.

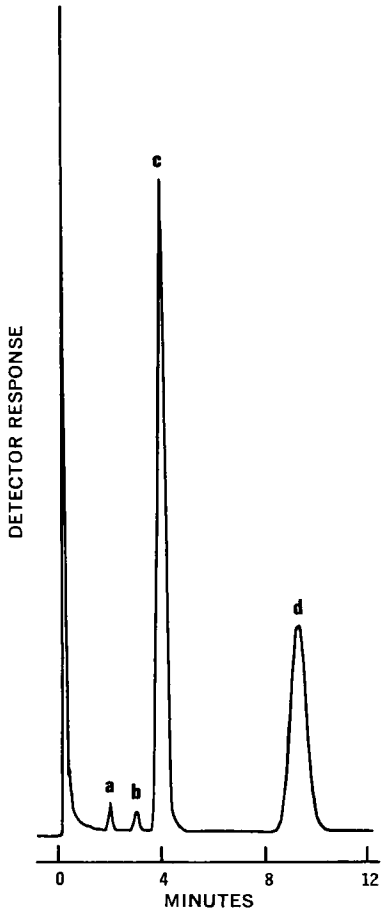


Figure 1—*Chromatogram of: (a) trifluoroacetylated epilincomycin, (b) trifluoroacetylated clindamycin B, (c) trifluoroacetylated clindamycin, and (d) hexacosane as internal standard.*

Calculations—The percent purity was calculated using the equation:

$$\% \text{ purity} = \frac{R_1}{R_2} \times \frac{W_2}{W_1} \times F \times 100 \qquad \text{(Eq. 1)}$$

where R_1 = peak area ratio of sample peak to internal standard peak, R_2 = peak area ratio of reference peak to internal standard peak, W_1 = weight in milligrams of sample, W_2 = weight in milligrams of reference, and F = dilution and conversion factor.

RESULTS AND DISCUSSION

Clindamycin Hydrochloride—Due to the polar groups on the clindamycin molecule, derivatization was necessary for GLC. GLC–mass spectrometry[4] indicated that trifluoroacetylated clindamycin chromatographs intact as the derivatized free base containing three trifluoroacetyl groups.

A chromatogram showing impurities in a research lot of clindamycin hydrochloride is shown in Fig. 1. When present, lincomycin appears between the epilincomycin and clindamycin B peaks and epiclindamycin appears on the tailing edge of the clindamycin peak. Impurity results (Table I) were obtained using peak area percent (without an internal standard), in which the total area of all peaks was assigned to be 100%. As can be seen in Table I, less measurement variation was obtained when the impurity was present in larger amount. GLC weight-response ratios for authentic samples of epilincomycin, epiclindamycin, and clindamycin B relative to clindamycin were determined to be approximately equal.

Linearity and complete extraction of clindamycin were obtained

[4] LKB 9000 mass spectrometer.

Table II—Precision of Replicate Samples of Three Clindamycin Formulations

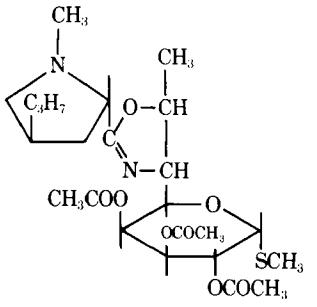
Sample, mg or ml	Clindamycin Base Found, % of Theory
150-mg Clindamycin Hydrochloride Capsules	
134.89	98.5
138.94	101.2
139.83	101.8
135.37	99.5
146.92	102.7
137.03	99.1
Average	100.5 *CV* 1.7%
150-mg/ml Sterile Solution of Clindamycin 2-Phosphate	
1.0	103.0
1.0	100.0
1.0	102.0
1.0	102.0
1.0	102.0
1.0	103.0
Average	102.0 *CV* 0.8%
Flavored Granules of Clindamycin 2-Palmitate	
616.71	100.8
719.90	103.8
755.28	103.0
865.65	102.4
881.77	103.8
790.61	101.4
Average	102.5 *CV* 1.2%

for the hard-filled capsule formulation over the range of ±50% of label. The coefficient of variation of the assay using six replicate samples was less than 2% (Table II). Correlation between this chromatographic procedure and bioassay was excellent. A chromatogram obtained using this procedure is shown in Fig. 1.

In earlier experiments with acetylated clindamycin, using a heavy load column at higher temperature (10% SE-30 column at 250–300°), a relatively small peak appeared on the chromatogram in addition to the large main peak. This small peak had a shorter retention time and tailed badly into the main peak, suggesting on-column degradation. To clarify this finding, the main chromatographic peak was collected in a glass capillary tube and reinjected. Again, the small peak was obtained in addition to the main peak, indicating that the small peak was due to on-column degradation and not to an impurity appearing because of better separation on the heavy load column.

GLC–mass spectroscopy of this degradation peak indicated a molecular weight 36 mass units less than derivatized clindamycin free base, with no evidence of chlorine isotope. The loss of 36 mass units apparently was due to the loss of hydrogen chloride from the molecule. Further characterization of the degradation peak was obtained by IR spectroscopy on a collected sample. IR results showed a loss of amide bands and the appearance of a C=N band when compared with the spectra of acetylated clindamycin. Structure VIII seems to satisfy the data for this on-column degradation peak.

Assay of clindamycin hydrochloride using the acetyl derivative was satisfactory, but separation of epilincomycin from epiclindamycin was not complete (Fig. 2). Higher temperature and longer



VIII

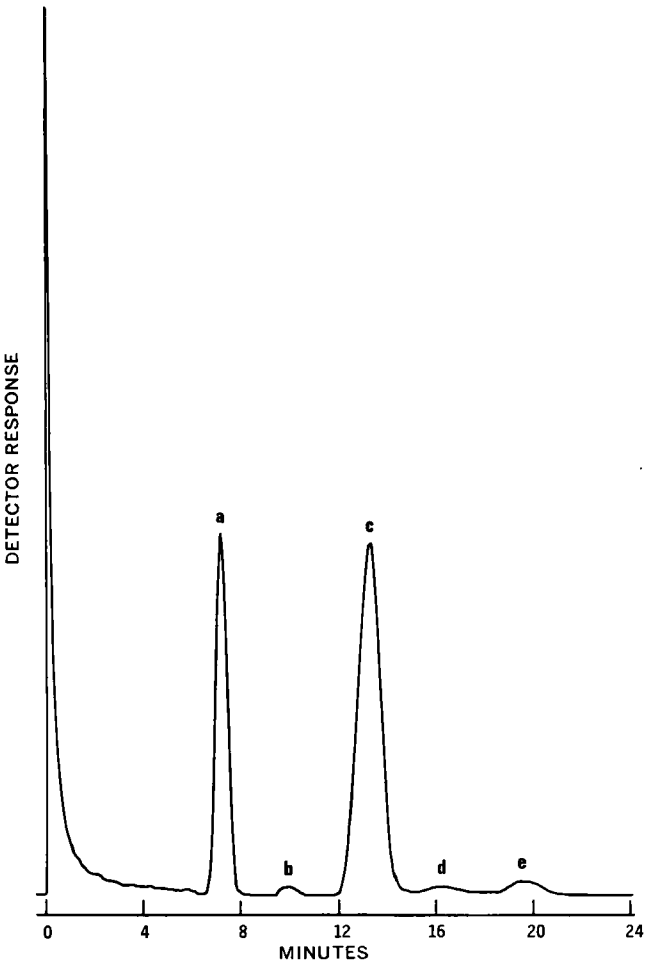


Figure 2—*Chromatogram of: (a) cholestane as internal standard, (b) acetylated clindamycin B, (c) acetylated clindamycin, (d) acetylated epiclindamycin and/or acetylated epilincomycin, and (e) acetylated lincomycin.*

heating time were required with the acetyl derivative procedure. In other experiments using a silylated derivative, clindamycin hydrochloride was not completely separated from lincomycin.

Clindamycin 2-Phosphate—Since this clindamycin ester could not be satisfactorily chromatographed even in a derivatized form, it was hydrolyzed to clindamycin using alkaline phosphatase enzyme, trifluoroacetylated, and then measured by GLC. The coefficient of variation obtained by assaying six replicate samples of sterile solution formulation was approximately 1% (Table II). Clindamycin hydrochloride was used as the reference in the assay instead of clindamycin 2-phosphate mainly because clindamycin 2-phosphate is quite hygroscopic, making it difficult to weigh. However, clindamycin 2-phosphate reference was used to demonstrate complete enzyme hydrolysis.

Impurities present in clindamycin hydrochloride can also be present in their ester form in clindamycin 2-phosphate and detected after hydrolysis (Fig. 1). In addition, it is possible for clindamycin 3-phosphate and clindamycin 4-phosphate to be formed in the synthesis from clindamycin hydrochloride. To determine the specificity of the assay in their presence, authentic samples of these possible impurities were carried through the assay procedure. Clindamycin 3-phosphate was only 37% hydrolyzed and clindamycin 4-phosphate was less than 1% hydrolyzed in the 2.5-hr incubation time of the procedure. Clindamycin hydrochloride, present as an impurity in clindamycin 2-phosphate, is removed in the chloroform wash of the procedure and can be assayed after concentration of the chloroform wash.

Clindamycin 2-Palmitate Hydrochloride—This ester was derivatized with acetic anhydride before GLC. Derivatizing agents such as trifluoroacetic anhydride, *n*-butyl boronic acid, and silylation reagents were more rapid but GLC was erratic. On some col-

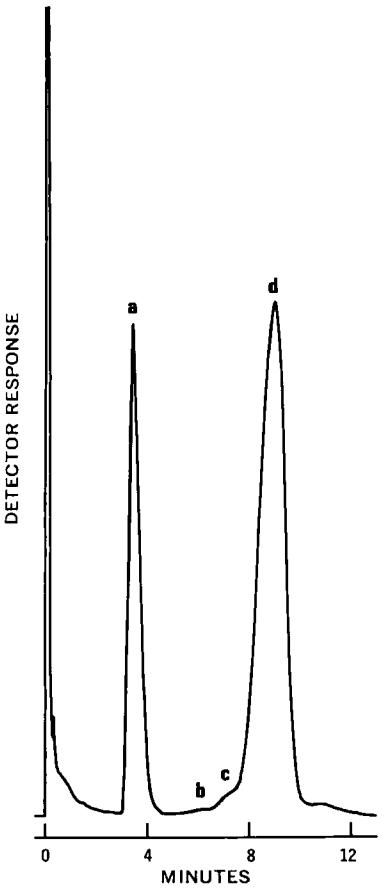


Figure 3—*Chromatogram of: (a) cholesteryl benzoate as internal standard, (b) acetylated clindamycin 4-palmitate, (c) acetylated clindamycin 3-palmitate and/or acetylated clindamycin B 2-palmitate, and (d) acetylated clindamycin 2-palmitate.*

umns a large shoulder appeared on the main chromatographic peak. Prior experience chromatographing acetylated clindamycin at elevated temperature suggested that acetylated clindamycin 2-palmitate may not chromatograph intact but as a cyclic structure, analogous to that proposed for the on-column degradation peak of acetylated clindamycin, and that the shoulder sometimes seen might be due to incomplete degradation to the cyclic compound. GLC–mass spectroscopy obtained on an acetylated clindamycin 2-palmitate peak showed a parent ion at *m/e* 711, indicating the presence of two acetyl groups and loss of hydrogen chloride. Again, no isotopic chlorine peaks were observed. Introduction of a derivatized clindamycin 2-palmitate sample by direct probe into the mass spectrometer produced a molecular ion of 747, showing that loss of HCl in using GLC–mass spectroscopy was due to the column and not the spectrometer. Since no impurity of this nature was detected in clindamycin 2-palmitate hydrochloride drug lots, the GLC peak obtained by on-column degradation of the derivatized ester was acceptable for quantitation. Assay of bulk drug or flavored granules gave a coefficient of variation of approximately 1.2% (Table II).

This compound also has several possible impurities which must be considered in the assay procedure. Clindamycin, clindamycin B 2-palmitate, clindamycin 3-palmitate, and clindamycin 4-palmitate are some impurities found in research lots of clindamycin 2-palmitate. As shown in Fig. 3, clindamycin 4-palmitate is completely separated from clindamycin 2-palmitate while clindamycin 3-palmitate appears as a shoulder on the front side of the clindamycin 2-palmitate peak. Clindamycin B 2-palmitate also appears as a shoulder on the front side; consequently, the assay procedure does not distinguish between these two impurities. The amount of clindamycin B 2-palmitate has been determined by hydrolyzing the sample with strong base, extracting, and then chromatographing, using the trifluoroacetyl derivative procedure as for clindamycin. The shoulder on the back side of the main peak in Fig. 3 is believed to be due to derivatized epiclindamycin 2-palmitate and/or epilincomycin 2-palmitate, analogous to that for acetylated clindamycin in Fig. 2.

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