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# A high-performance liquid chromatographic assay for clindamycin phosphate and its principal degradation product in bulk drug and formulations

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**Abstract**: A high-performance liquid chromatography method has been developed for the analysis of clindamycin phosphate and clindamycin, the principal degradation product. The method is quantitative, precise and is able to separate a variety of closely related molecules. The method has been applied to bulk drug, topical and sterile solutions, and experimental cream, lotion and gel formulations. The method gives results that are in good agreement with the official gas chromatographic method but is much less time-consuming.

**Keywords**: *Clindamycin phosphate bulk drug and formulations; stability-indicating assay; high-performance liquid chromatography.*

## Introduction

Clindamycin phosphate (I) is the 2-phosphate ester of clindamycin and is available as a sterile solution (Cleocin Phosphate™ Sterile Solution, The Upjohn Company, Kalamazoo, MI) and a topical solution (Cleocin T™ Topical Solution, The Upjohn Company, Kalamazoo, MI). The sterile solution is indicated for the treatment of serious infections caused by susceptible anaerobic bacteria and by susceptible strains of streptococci, pneumococci and staphylococci. The topical solution is useful for the treatment of acne vulgaris.

Analysis of I is frequently accomplished by enzymatic hydrolysis of the phosphate ester, derivatization of the resultant clindamycin and quantitation by gas chromatography using clindamycin as the reference standard. In most cases the liberated clindamycin must be extracted from aqueous solutions before derivatization.

This report details a reversed-phase HPLC procedure that avoids the hydrolysis, extraction and derivatization steps required for the GC procedure. Samples are prepared by simple dilution and quantitated at 210 nm. Since this method uses clindamycin phosphate as the reference standard, a standard was characterized to support this methodology. This method was applied to bulk drug, sterile solution, topical solution and experimental lotion, gel and cream formulations.

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The official assay for **I** [1, 2] employs hydrolysis of the phosphate ester by the action of intestinal alkaline phosphatase, extraction of the resultant free clindamycin into an organic solvent, evaporation of the solvent and derivatization with trifluoroacetic anhydride. The derivative is then analysed using SE-30 on Diatoport S (80/100 mesh) and flame ionization detection. Losses due to incomplete hydrolysis, extraction or derivatization may lead to low results. Furthermore, the procedure is quite time-consuming.

High-performance liquid chromatography has been used in several instances for the direct analysis of **I**. Morozowich and Williams [3] used a high capacity triethylaminoethyl cellulose column, a mobile phase consisting of 0.25 M boric acid at pH 8.8 and detection at 254 nm to quantitate **I**. Since the analyte has a very small absorptivity at 254 nm, this method lacked sensitivity. In a later work, Brown [4] employed refractive index detection and a microparticulate octadecylsilane column to analyse **I**. While this method was superior to previously reported HPLC methods, it suffered from the instabilities and sensitivity limits associated with refractive index detectors.

To avoid problems of low absorptivity at 254 nm several investigators have used end absorption (below 220 nm) for the analysis of clindamycin and clindamycin phosphate. Clindamycin has been analysed at 214 nm using an ion pair (sodium pentane sulphonate) system for the separation [5]. At the 1985 Pittsburgh Conference in New Orleans, P. A. Asmus and J. B. Landis described a method that used detection at 215 nm and a reversed-phase system to quantitate clindamycin phosphate and its degradation products. Gradient elution was used to resolve all potential degradation products within a reasonable chromatographic run time. In this study dimethyloctylamine was used to suppress peak tailing.

In a recent study of the stability of clindamycin hydrochloride and **I**, workers used ion pair formation with dioctyl sodium sulphosuccinate and detection at 254 nm [6]. Lack of absorptivity at this wavelength was not a limiting factor. This system, however, did not allow for the simultaneous analysis of **I** and the principal degradation product clindamycin.

## Experimental

### *Materials*

Clindamycin-2-phosphate and related materials were obtained from The Upjohn Company. All other reagents and solvents were analytical reagent grade and were used without further purification. A lot of **I** was characterized to serve as a reference standard. The characterization is described in a later section.

### *Apparatus*

A modular chromatographic system was employed consisting of a reciprocating single piston pump (Altex 110A with pulse dampener), an automated loop injector (20 μl loop), a microparticulate octasilane column (Zorbax $C_8$, 250 × 4.6 mm, DuPont), and a variable wavelength detector (LDC Spectro-Monitor III) at 210 nm. Data were collected and processed by a laboratory data system (DEC) developed by The Upjohn Company [7].

### *Mobile phase*

The mobile phase used in the analysis of bulk drug and dosage forms was prepared by first dissolving 10.54 g of potassium phosphate monobasic in 775 ml of deionized water.

After adjusting to pH 2.5 with phosphoric acid, 225 ml of acetonitrile was added and mixed well. The final solution was filtered through a 0.2-μm nylon filter.

*Characterization of reference standard*

A lot of **I** was characterized by evaluation of impurity content (HPLC), residual solvents by gas chromatography and water by Karl Fischer titration. During this process, equivalence of chromatographic response factors was assumed. Water content was found to be 3.4% which is consistent with the monohydrate form. This form was shown to be stable at relative humidities (at 25°C) from 12 to 81%. The purity of 799 μg $mg^{-1}$ was assigned by subtracting the minor components and correcting for the phosphate composition. This purity is expressed as clindamycin base equivalents.

*Characterization of clindamycin-B-phosphate*

In most bulk drug samples a peak of substantial size appeared at about 0.5 of the retention time of **I**. Since Clindamycin B is a normal component of clindamycin it is reasonable to suspect that this peak could be clindamycin-B-phosphate. To test this hypothesis, a sample of drug known to be enriched with clindamycin-B-phosphate (as determined by gas chromatography) was injected into this system. The fraction eluting at a relative retention time of 0.55 was collected using a preparative scale octasilane column. After evaporation of the solvent, the residue was analysed by chemical ionization (ammonia) mass spectrometry. The fragmentation pattern obtained was displaced by 14 units which is consistent for clindamycin-B-phosphate, the difference in structure being a methylene group.

*Analytical procedure*

An accurately weighed (or measured) quantity of sample containing the equivalent of 20 mg clindamycin (about 24 mg of **I**) was transferred to a suitable container. An accurately measured quantity of internal standard (usually 25.0 ml of *p*-hydroxyacetophenone, 40 mg/l in mobile phase) was added followed by a volume of mobile phase (usually 75 ml). The container was stoppered and shaken until the sample dissolved. For the sterile solution the *p*-hydroxyacetophenone internal standard was replaced by a solution of methylparaben (60 mg/l of mobile phase). Also, the sterile solution was diluted 50-fold initially with water.

Aliquots (20 μl) of the sample preparations and standards were subjected to chromatographic analysis with the concentrations of **I** and clindamycin being determined by peak area or peak height ratios.

*Recovery studies*

Accurately measured quantities of **I** or clindamycin hydrochloride were added to the appropriate amount of placebo and then analysed by the procedure outlined above.

## Results and Discussion

The effects of acetonitrile concentration, buffer concentration and mobile phase pH (as measured before the addition of acetonitrile) on capacity factor were evaluated for **I**, clindamycin and the internal standard, *p*-hydroxyacetophenone. The effect of acetonitrile concentration is shown in Fig. 1. As expected, the retention of all three components changed dramatically with relatively small changes in acetonitrile concen-

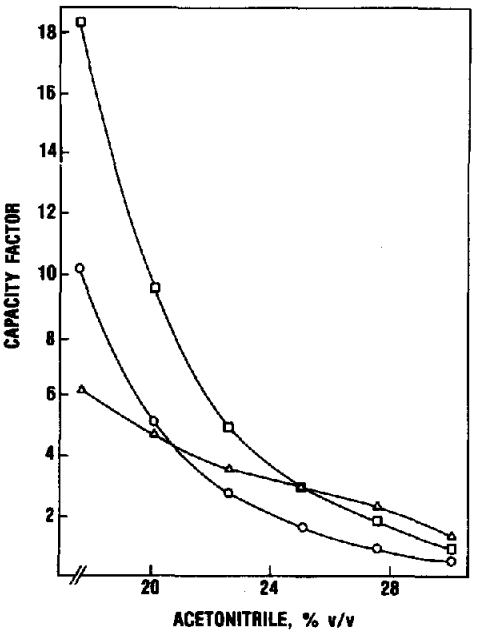


**Figure 1**
Effect of acetonitrile concentration on capacity factors for clindamycin-2-phosphate, O, clindamycin, □; and *p*-hydroxyacetophenone, △. pH held constant at 2.5 with a phosphate concentration of 0.08 M.

tration. A concentration of 22.5% (v/v) was chosen as a compromise that presented adequate resolution while keeping total elution time relatively low. The effect of mobile phase pH on retention is shown in Fig. 2. Between pH 2.5 and 4.5, the retention of all components changed very little. The pH value of 2.5 was chosen on peak shape considerations. Values below pH 2.5 were not investigated because of potential column stability problems. At higher pH values (greater than 5) the retention of clindamycin became too long. The effect of buffer concentration (Fig. 3) is minimal over the range of buffer concentrations explored.

A variety of related molecules were examined with the final analytical system. Relative retention times for these compounds are found in Table 1. The only potential interferences arise from the possible overlap of the internal standard, *p*-hydroxyacetophenone and clindamycin-3-phosphate. Since this is of significance only for the analysis of bulk drug, several lots were examined closely for the presence of clindamycin-3-phosphate. No peaks were observed in the region of elution. If desired, the internal standard could be left out and quantitation could be performed by an external standard technique. Two other degradation products, lincomycin and lincomycin-2-phosphate, do not interfere. While these peaks overlap, they elute well before any compounds or interest.

Typical chromatograms of the sterile solution and topical solution (Fig. 4) show that the formulation ingredients do not interfere with peak I. In most cases the internal standard elutes in a clear region of the chromatogram. In the case of the sterile solution, the preservative benzyl alcohol overlaps with the internal standard *p*-hydroxyacetophenone. Consequently, a different internal standard, methyl paraben, was employed. Recovery of the drug from spiked placebos was excellent, as shown in Table 2. The results obtained by peak height measurements were equivalent to those obtained by peak area measurements. The precision of the assay was determined by multiple analyses of a

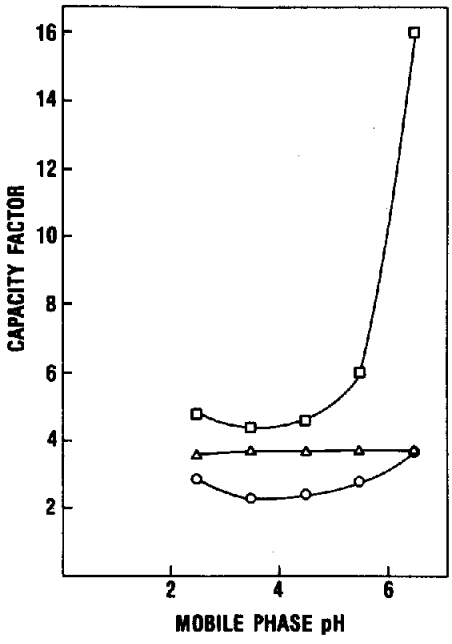


**Figure 2**
Effect of mobile phase pH on capacity factor for compounds listed in Fig. 1. Acetonitrile concentration held constant at 22.5% and phosphate concentration at 0.08 M.

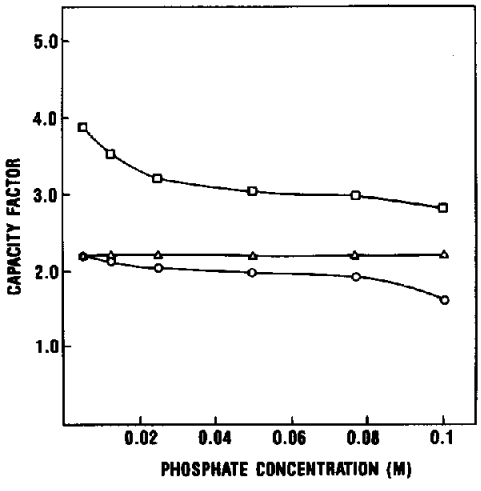


**Figure 3**
Effect of phosphate concentration on capacity factor for compounds listed in Fig. 1. Acetonitrile was held at 22.5% with a pH of 2.5

single lot of bulk drug and each formulation. The relative standard deviation was typically less than 2.0%, as shown in Table 3.

Spike recovery studies for clindamycin hydrochloride were performed to demonstrate the utility of this assay for quantifying the principal degradation product of **I**. The results (Table 4) indicate that this assay is suitable for that purpose. The precision of the assay, expressed as relative standard deviation, was less than 8% at levels of clindamycin ranging from 0.09 to 1.4 mg $ml^{-1}$ (or gram) (Table 5).

The equivalence of the HPLC assay to the gas chromatographic assay was evaluated by comparisons of results of the two methods obtained on common samples. Comparison of

**Table 1**
Relative retention times of related molecules, preservatives, and internal standards

| Compound | Relative retention time |
|---|---|
| Lincomycin B HCl | 0.33 |
| Lincomycin-2-phosphate | 0.35 |
| Lincomycin HCl | 0.40 |
| Clindamycin B-2-phosphate | 0.55 |
| (Clindamycin-2-phosphate) | (1.00) |
| Benzyl alcohol | 1.20 |
| Clindamycin-3-phosphate | 1.24 |
| *p*-Hydroxyacetophenone | 1.27 |
| Clindamycin-4-phosphate | 1.61 |
| Clindamycin HCl | 1.69 |
| Methylparaben | 2.46 |
| Benzaldehyde | 3.11 |

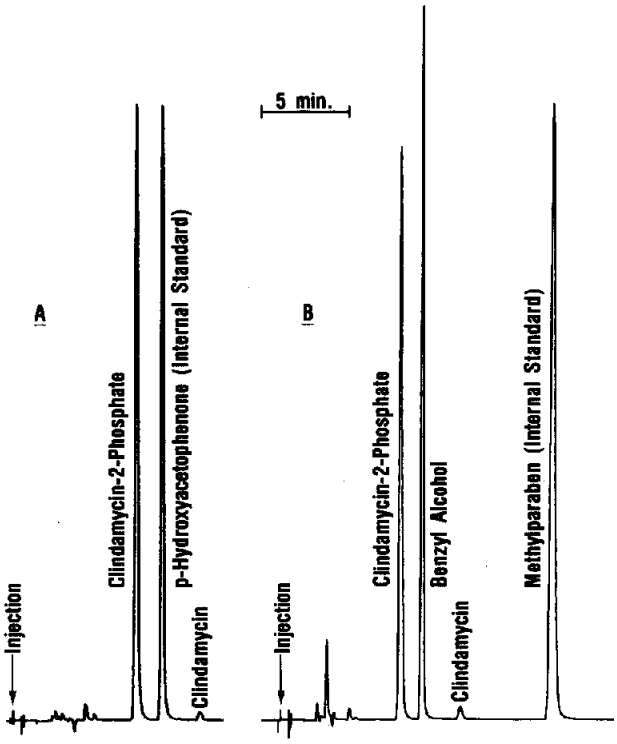


**Figure 4**
Typical chromatograms for sample preparations of the topical solution (A) and sterile solution (B). Aged samples were used to show position of clindamycin peak.

**Table 2**
Recovery of clindamycin-2-phosphate from spiked placebo

| | Clindamycin base equivalents | | | | | |
|---|---|---|---|---|---|---|
| | Area | | | Height | | |
| Added (mg) | Found (mg) | | R (%) | Found (mg) | | R (%) |
| A. Bulk drug (linearity study, no placebo added) | | | | | | |
| 14.2 | 14.3 | | 100.7 | 14.4 | | 100.7 |
| 17.4 | 17.5 | | 100.6 | 17.4 | | 100.0 |
| 20.4 | 20.5 | | 100.5 | 20.6 | | 101.0 |
| 22.5 | 22.5 | | 100.0 | 22.4 | | 99.6 |
| 25.3 | 25.2 | | 99.6 | 25.3 | | 100.0 |
| | | $\bar{x}$ | 110.3 | | $\bar{x}$ | 100.3 |
| B. Sterile solution* | | | | | | |
| 10.4 | 10.4 | | 100.0 | 10.6 | | 102.0 |
| 17.3 | 17.5 | | 101.2 | 17.4 | | 100.6 |
| 20.8 | 20.8 | | 100.0 | 20.9 | | 100.5 |
| 24.2 | 24.2 | | 100.0 | 24.1 | | 99.6 |
| 27.7 | 27.8 | | 100.4 | 27.3 | | 98.6 |
| | | $\bar{x}$ | 100.3 | | $\bar{x}$ | 100.3 |
| C. Topical solution† | | | | | | |
| 13.4 | 13.5 | | 100.7 | 13.7 | | 102.2 |
| 16.8 | 16.8 | | 100.0 | 16.9 | | 100.6 |
| 20.2 | 20.0 | | 99.0 | 20.1 | | 99.5 |
| 23.5 | 23.3 | | 99.1 | 23.3 | | 99.1 |
| 26.9 | 26.6 | | 98.9 | 26.6 | | 98.9 |
| | | $\bar{x}$ | 99.5 | | $\bar{x}$ | 100.1 |
| D. Lotion‡ | | | | | | |
| 18.0 | 18.5 | | 102.8 | 18.3 | | 101.6 |
| 21.0 | 21.2 | | 100.9 | 21.0 | | 100.0 |
| 24.0 | 24.5 | | 102.1 | 24.3 | | 101.2 |
| 27.0 | 26.9 | | 99.6 | 27.3 | | 101.1 |
| 30.0 | 29.7 | | 99.0 | 30.0 | | 100.0 |
| | | $\bar{x}$ | 100.9 | | $\bar{x}$ | 100.8 |
| E. Gel‡ | | | | | | |
| 18.0 | 17.9 | | 99.4 | 18.1 | | 100.6 |
| 21.0 | 21.1 | | 100.5 | 21.1 | | 100.5 |
| 24.0 | 24.2 | | 100.8 | 24.1 | | 100.4 |
| 27.0 | 26.5 | | 98.1 | 27.0 | | 100.0 |
| 30.0 | 29.5 | | 98.3 | 29.9 | | 99.7 |
| | | $\bar{x}$ | 99.4 | | $\bar{x}$ | 100.2 |
| F. Cream‡ | | | | | | |
| 14.5 | 14.7 | | 101.4 | 14.6 | | 100.7 |
| 17.0 | 17.2 | | 101.2 | 17.1 | | 100.6 |
| 20.3 | 20.5 | | 101.0 | 20.3 | | 100.0 |
| 23.0 | 23.0 | | 100.0 | 22.8 | | 99.1 |
| 25.1 | 24.9 | | 99.2 | 24.8 | | 98.8 |
| | | $\bar{x}$ | 100.6 | | $\bar{x}$ | 99.8 |

*A 2.0 ml sample gives a final sample preparation containing 21.0 mg of clindamycin base equivalents. This is equivalent to 150 mg $ml^{-1}$ in the product.

†A 2.0 ml sample contains 20 mg of clindamycin base equivalents at 100% of label.

‡A 2.0 g sample contains 20 mg of clindamycin base equivalents at 100% of label.

**Table 3**
Precision of assays for replicate sample preparations

| Material | Mean* | Rel. std. dev. Area (%) | Height (%) | *n* |
|---|---|---|---|---|
| Bulk drug | 788 μg/mg | 0.4 | 0.7 | 15 |
| Sterile solution | 154 mg/ml | 0.6 | 0.8 | 10 |
| Topical solution | 10.1 mg/ml | 1.0 | 0.8 | 10 |
| Lotion | 10.5 mg/g | 1.8 | 1.6 | 9 |
| Gel | 10.0 mg/g | 0.8 | 0.7 | 9 |
| Cream | 10.4 mg/g | 0.6 | 0.7 | 10 |

* Expressed as free base equivalents.

**Table 4**
Recovery of clindamycin free base from spiked placebo formulations

| Added (mg)* | Peak area Found (mg) | R (%) | Peak height Found (mg) | R (%) |
|---|---|---|---|---|
| A. Sterile solution | | | | |
| 0.20 | 0.21 | 104 | 0.22 | 107 |
| 0.40 | 0.41 | 103 | 0.41 | 103 |
| 0.60 | 0.62 | 103 | 0.62 | 103 |
| 0.80 | 0.83 | 104 | 0.83 | 104 |
| 1.00 | 1.05 | 105 | 1.04 | 104 |
| B. Topical solution† | | | | |
| 0.20 | 0.21 | 105 | 0.21 | 105 |
| 0.41 | 0.40 | 98 | 0.40 | 98 |
| 0.61 | 0.58 | 95 | 0.59 | 97 |
| 0.82 | 0.81 | 99 | 0.81 | 99 |
| 1.02 | 1.01 | 99 | 1.02 | 100 |
| C. Lotion | | | | |
| 0.21 | 0.24 | 114 | 0.22 | 105 |
| 0.52 | 0.53 | 102 | 0.52 | 100 |
| 0.83 | 0.83 | 100 | 0.82 | 99 |
| D. Gel | | | | |
| 0.24 | 0.24 | 100 | 0.25 | 104 |
| 0.49 | 0.42 | 86 | 0.46 | 94 |
| 0.74 | 0.71 | 96 | 0.71 | 96 |
| 0.98 | 0.95 | 97 | 0.96 | 98 |
| 1.22 | 1.16 | 95 | 1.18 | 97 |
| E. Cream | | | | |
| 0.20 | 0.20 | 100 | 0.21 | 105 |
| 0.41 | 0.40 | 100 | 0.42 | 102 |
| 0.61 | 0.61 | 100 | 0.62 | 102 |
| 0.82 | 0.80 | 98 | 0.80 | 100 |
| 1.02 | 1.05 | 103 | 1.01 | 98 |

* mg/added per 2.0 ml or of samples after dilution.
† mg/added per 2.0 ml or 2.0 g of sample. 1.0 mg is equal to 5% w/w of the clindamycin base equivalents.

**Table 5**
Precision of assay for free base for replicate sample preparations

| Product | Mean | Rel. std. dev. Area (%) | Height (%) | *n* |
|---|---|---|---|---|
| Sterile solution | 1.4 mg/ml | 6.5 | 6.0 | 9 |
| Topical solution | 0.18 mg/ml | 2.9 | 2.9 | 10 |
| Lotion | 0.23 mg/ml | 5.6 | 2.8 | 12 |
| Gel | 0.09 mg/g | 7.3 | 5.2 | 10 |
| Cream | 0.13 mg/g | 3.9 | 2.6 | 9 |

**Table 6**
Comparison of assay results for bulk drug

| Lot | HPLC ($\mu g\ mg^{-1}$)* | GC ($\mu g\ mg^{-1}$)* | Difference (%) |
|---|---|---|---|
| A | 787 | 775 | +1.5 |
| B | 790 | 787 | +0.4 |
| C | 802 | 792 | +1.2 |
| D | 808 | 782 | +3.2 |
| E | 790 | 783 | +0.9 |
| F | 778 | 789 | −1.4 |
| G | 781 | 780 | +0.1 |
| H | 791 | 774 | +2.1 |

*Duplicate determinations.

**Table 7**
Comparison of assay results for topical solutions

| Lot | HPLC ($mg\ ml^{-1}$)* | GC[a] ($mg\ ml^{-1}$) | Difference (%) |
|---|---|---|---|
| A | 10.32 | 10.21 | +1.1 |
| B | 9.93 | 9.76 | +1.7 |
| C | 10.27 | 10.32 | −0.5 |
| D | 10.09 | 10.21 | −1.2 |
| E | 10.01 | 10.04 | −0.2 |
| F | 10.44 | 10.22 | +2.1 |
| G | 10.21 | 9.99 | +2.1 |
| H | 9.89 | 9.96 | −0.7 |

*Average of two determinations.

**Table 8**
Comparison of results for sterile solution

| Lot* | HPLC ($mg\ ml^{-1}$)† | GC ($mg\ ml^{-1}$) | Difference (%) |
|---|---|---|---|
| A | 140 | 138 | +1.4 |
| B | 139 | 139 | — |
| C | 137 | 137 | — |
| D | 137 | 138 | −0.7 |
| E | 138 | 135 | +2.2 |
| F | 139 | 136 | +2.2 |
| G | 138 | 138 | — |

*All samples were older than three years.
†Duplicate determination (average).
‡Single determination.

bulk drug lots is shown in Table 6. In most cases the liquid chromatographic assay gave results slightly higher than the gas chromatographic method. Even though these results were obtained in different laboratories, the trend is observable. Similar results for the topical and sterile solutions are found in Tables 7 and 8. To test further the equivalence of the methods, data collected on single lots of bulk drug, topical solution and sterile solution were compared. The gas chromatographic data were collected over a number of

**Table 9**
Comparison of reported method and gas chromatographic results on common lots

| Method | Result | *n* | Sx | RSD (%) |
|---|---|---|---|---|
| A. Bulk drug | | | | |
| HPLC | 804 μg $mg^{-1}$ | 12 | 4.0 | 0.5 |
| GC-A | 785 μg $mg^{-1}$ | 20 | 5.5 | 0.7 |
| GC-B | 776 μg $mg^{-1}$ | 8 | 12.4 | 1.6 |
| GC-C | 775 μg $mg^{-1}$ | 42 | 17.1 | 2.2 |
| B. Topical solution | | | | |
| HPLC | 10.1 mg $ml^{-1}$ | 12 | 0.065 | 0.6 |
| GC-A | 9.7 mg $ml^{-1}$ | 20 | 0.20 | 2.1 |
| GC-B | 10.0 mg $ml^{-1}$ | 20 | 0.26 | 2.6 |
| GC-C | 9.9 mg $ml^{-1}$ | 20 | 0.13 | 1.3 |
| C. Sterile solution | | | | |
| HPLC | 152 mg $ml^{-1}$ | 9 | 0.76 | 0.5 |
| GC-A | 152 mg $ml^{-1}$ | 25 | 5.5 | 3.6 |
| GC-B | 151 mg $ml^{-1}$ | 21 | 3.9 | 2.6 |
| GC-C | 148 mg $ml^{-1}$ | 21 | 3.3 | 2.2 |

**Table 10**
Statistical comparison of HPLC assay to gas chromatographic assay

| | HPLC | GC-A | GC-B | GC-C |
|---|---|---|---|---|
| A. Bulk drug | | | | |
| HPLC | − | + | + | + |
| GC-A | + | − | − | + |
| GC-B | + | − | − | − |
| GC-C | + | + | − | − |
| B. Topical solution | | | | |
| HPLC | − | + | − | − |
| GC-A | + | − | + | − |
| GC-B | − | + | − | − |
| GC-C | − | + | − | − |
| C. Sterile solution | | | | |
| HPLC | − | − | − | + |
| GC-A | − | − | − | + |
| GC-B | − | − | − | + |
| GC-C | + | + | + | − |

+ Significantly different at the 95% confidence level using the method of simultaneous confidence limits for mean differences.
− Not significantly different.

days in three different laboratories (A, B, C) while the liquid chromatography results were obtained in the authors' laboratories. These data are summarized in Table 9. The results were compared for statistical significance using simultaneous limits for mean differences. These data are shown in Table 10. In all three instances the liquid chromatographic method gave results statistically different from those obtained by the gas chromatographic method for the bulk drug. In the cases of topical and sterile solutions the liquid chromatographic method gave results statistically equivalent to the gas chromatographic method in two out of three cases. Other formulations could not be used for this comparison since the gas chromatographic method gave interfering peaks.

Even though the proposed method gives slightly higher results than the gas chromatographic procedure in many cases, it is acceptable in terms of recovery and precision. Since each method produces results relative to the reference standard, the differences may be attributable to differences in purity determination for the individual standards. Clindamycin hydrochloride is used as a reference standard for the GC assay while clindamycin phosphate is used for the HPLC assay. Furthermore, losses in any step of the gas chromatographic assay would lead to lower results.

## Conclusion

The liquid chromatographic assay has been shown to give quantitative recovery and acceptable precision. It is specific and suitable for monitoring the degradation product clindamycin. It is experimentally simple, requiring only simple dilution, and gives reasonable agreement with the gas chromatographic method.

*Acknowledgements*: The contributions of D.H. Hassing are acknowledged. The assistance of M. E. Gerger in the statistical analysis is also appreciated.

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