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# Development and validation of a gradient HPLC method for the determination of clindamycin and related compounds in a novel tablet formulation

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**Abstract**

A gradient reversed-phase HPLC method was developed and validated for potency, content uniformity, and impurity determinations for a novel tablet formulation containing clindamycin. The assay utilized UV detection at 214 nm and a Waters Xterra $RP_{18}$ column (4.6 mm × 100 mm, 3.5 μm). The mobile phases were comprised of pH 10.5, 10 mM carbonate buffer and acetonitrile.

Validation experiments were performed to demonstrate specificity, linearity, accuracy (i.e., average recovery from the formulation), precision (i.e., repeatability), limit of quantitation (LOQ), and robustness (i.e., sample solution stability and buffer pH effects on specificity). The assay was shown to be specific for clindamycin, several impurities, and triethyl citrate, a retained excipient that was present in the dosage form. The assay was proved linear (concentration versus peak area) for clindamycin and several select impurities over the ranges of 70–130% and 0.1–5%, respectively. UV relative response factors were determined for the impurities from the linearity data. The accuracy of clindamycin at the targeted assay concentration was 99.2% ($n = 3$; precision = 0.12%, R.S.D.); accuracy for lincomycin, a structurally related impurity, was 97.4% ($n = 3$; precision = 3.5%, R.S.D.) at 0.1% of the targeted assay concentration. By demonstrating an acceptable degree of precision for lincomycin at this level, the LOQ was shown to be no higher than 0.1%. The chromatography was virtually unaffected over a mobile phase buffer pH range spanning 0.4 pH units. Sample solutions were stable for 72 h under ambient conditions.
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*Keywords:* Reversed-phase chromatography; Clindamycin; Content uniformity; Potency; Impurities; Dosage form

## 1. Introduction

Clindamycin (including the HCl salt and other forms) (Fig. 1 and Table 1) is a common antibiotic that is marketed for the treatment of certain Gram-positive bacterial infections. Pfizer Inc. recently investigated clindamycin in a novel and proprietary tablet formulation.

An in-house method adapted from Ref. [2] was found to be unsuitable for use with the new formulation, because triethyl citrate (a tablet excipient) and degradation product A (an impurity resulting from exposure of clindamycin to one of the excipients, formed over time under certain conditions) interfered with other clindamycin-related impurities.

This prompted a review of the literature, which showed that several HPLC methods have been developed over the years for clindamycin and clindamycin-related impurities [1–8]. Some of these relied upon ion-pair reagent in the mobile phase to effect the separation; therefore, they were not considered for this effort because of the inherent instability and long equilibration times often associated with such methods. Other efforts were concerned with the determination of clindamycin in biological matrices, without regard for related substances [7,8]. Orwa et al. attempted to utilize *USP* [9] and *Ph. Eur.* [10] methods to separate clindamycin and related components, with unsatisfactory results. Instead, they developed a novel isocratic separation that utilized UV detection [11,12]. More recently, the Ph. Eur. related substances test was changed to an isocratic HPLC method run under near-neutral conditions [13]. Although this was considered an advancement over previous technology, we found that the degradation product and triethyl citrate were either not well resolved from, or coeluted with, other clindamycin-related

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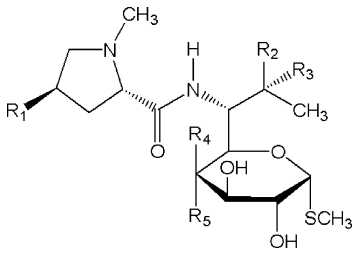


Fig. 1. Structure of the clindamycin core molecule.

Table 1
Structural information for clindamycin and related components

| Compound name | R1 | R2 | R3 | R4 | R5 |
|---|---|---|---|---|---|
| Clindamycin | $CH_2CH_2CH_3$ | Cl | H | OH | H |
| 7-Epiclindamycin | $CH_2CH_2CH_3$ | H | Cl | OH | H |
| Clindamycin B | $CH_2CH_3$ | Cl | H | OH | H |
| 4-Desoxy-4-α-chloroclindamycin B | $CH_2CH_3$ | Cl | H | H | Cl |
| Lincomycin | $CH_2CH_2CH_3$ | H | OH | OH | H |
| 7-Epilincomycin | $CH_2CH_2CH_3$ | OH | H | OH | H |

impurities under these conditions. In developing new conditions, a method capable of eluting a wide range of compounds of different polarities, with excellent efficiency and good band spacing, was desired. This was considered necessary because of the developmental nature of the formulation and a lack of familiarity with the degradation profile. Gradient elution represented the greatest chance for success.

This document describes the development and validation of a sensitive, selective, and relatively rapid gradient HPLC method for potency, content uniformity, and impurity testing for the clindamycin formulation. The method represents an alternative set of conditions that could prove useful under certain circumstances, either as an investigative tool or as an internal release test, depending on the components and interferences present in the sample.

## 2. Experimental

### 2.1. Materials and reagents

HPLC grade acetonitrile and water were obtained from Burdick and Jackson (Muskegon, MI, USA) and EMScience (Gibbstown, NJ, USA), respectively. Concentrated hydrochloric acid and anhydrous potassium carbonate were both obtained from Mallinckrodt (Paris, KY, USA). Triethyl citrate (TEC) was obtained from Morflex (Greensboro, NC, USA). The following reference materials were obtained in-house from Pfizer (Kalamazoo, MI, USA): clindamycin HCl (monohydrate) reference standard, clindamycin HCl resolution standard (a mixture containing clindamycin HCl and related substances), and lincomycin HCl (monohydrate) reference standard. The following authentic samples were also obtained in-house from Pfizer: clindamycin drug substance, 7-epilincomycin HCl, 7-epiclindamycin HCl, and degradation product A. The Pfizer Pharmaceutical R&D group in Kalamazoo provided a placebo mixture containing all of the formulation excipients in the proper amounts.

Table 2
Gradient program for clindamycin assay

| Time (min) | MP A:MP B |
|---|---|
| 0 | 75:25 |
| 3.5 | 75:25 |
| 18.5 | 60:40 |
| 19.5 | 60:40 |
| 19.6 | 75:25 |
| 24 | 75:25 |

### 2.2. HPLC apparatus and operating conditions

The chromatograph consisted of a Summit System from Dionex (Sunnyvale, CA, USA) comprised of a P-680 binary gradient pump and ASI-100 autosampler, and a Model 2487 variable wavelength UV detector from Waters (Milford, MA, USA) set at 214 nm. An XTerra $RP_{18}$ column (4.6 mm × 100 mm, 3.5 μm particle size) from Waters with in-line pre-filter was used for the separation. (The XTerra column utilized a polymer/silica "hybrid" solid support with a C18 bonded phase.) The injection volume and flow rate were 15 μL and 1.0 mL/min, respectively. Except where otherwise stated, 10 mM carbonate buffer was prepared by dissolving 1.38 ± 0.10 g potassium carbonate (anhydrous) in 1000 mL of water; the pH was adjusted to 10.5 using concentrated hydrochloric acid. The composition of mobile phase A was 90:10 carbonate buffer:acetonitrile. The composition of mobile phase B was 20:80 carbonate buffer:acetonitrile. Six to eight milligrams of sodium nitrate was added to each liter of mobile phase B. The gradient program is provided in Table 2. DryLab 2000 Plus®, a chromatography modeling software package from LC Resources (Walnut Creek, CA, USA) (acquired by Rheodyne (Rohnert Park, CA, USA)), was used during the development of the separation.

### 2.3. Method development and specificity experiments

For method development and specificity testing, a resolution mixture containing clindamycin at about 3.5 mg/mL and smaller quantities of impurities was prepared in mobile phase A. Solutions of individual impurities were also prepared and injected in cases where authentic impurity supplies were available. Formulation placebo samples were also prepared to ensure separation of excipients from peaks of interest.

### 2.4. Linearity

For the linearity experiments, solutions of clindamycin were prepared at five concentrations, spanning a range of 70–130% of the target clindamycin assay concentration. Likewise, solutions of lincomycin, 7-epilincomycin, 7-epiclindamycin, clindamycin B, and degradation product A were prepared at five concentrations, spanning a range of about 0.1–5% of the target clindamycin assay concentration.

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