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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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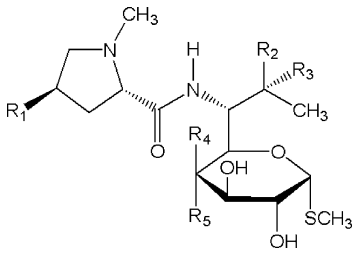

Fig. 1. Structure of the clindamycin core molecule.

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.

2.5. Accuracy, precision, and limit of quantitation (LOQ)

Accuracy, precision, and LOQ assessments were combined into a single set of experiments. Known quantities of clindamycin and lincomycin were combined with the proper amount of placebo mix to create three separate samples. These samples were designed to mimic the tablet formulation, and were prepared in the same way that actual tablets were prepared.

2.6. Robustness

For the robustness experiment related to specificity, two different 10 mM carbonate buffer solutions were prepared. The pH of one solution was adjusted to 10.3, while the pH of the other was adjusted to 10.7 (this range is ±0.2 pH units from nominal). From these two solutions, four mobile phase solutions were prepared. One set of mobile phases (A and B) was prepared using the pH 10.3 buffer, while the other set was prepared using the pH 10.7 buffer. A resolution mixture containing most of the peaks of interest was injected onto the chromatograph using each set of mobile phases.

For the assessment of sample solution stability, the clindamycin accuracy solutions were stored for 72 h under both ambient and refrigerated conditions and injected versus fresh standard preparations.

2.7. Preparation of tablet samples

For the composite tablet (potency and impurities) assay, 10 tablets were ground fine using a mortar and pestle. Grind containing an amount of clindamycin equivalent to a single tablet was then transferred to a 200 mL volumetric flask. Fifty milliliters of a 50:50 mixture of acetonitrile and 0.1N HCl was added; the flask was capped and shaken vigorously for 30 min. The flask was diluted to volume with pH 10.5 buffer, and the solution was gently mixed. An aliquot of the solution was centrifuged and filtered prior to injection. Single whole tablets were also subjected to this procedure for content uniformity determinations. This scheme was found to be optimal for both disintegrating whole tablets and dissolving the drug. It also resulted in a sample solution that approximated the initial mobile phase conditions, which was important for preserving the peak shape of early eluting components.

3. Results and discussion

3.1. Method development and specificity

From previous experience it was known that clindamycin, with a pK_a of about 7.6 [14], was poorly retained under most reversed-phase conditions. Ion-pair reagent had been used in the mobile phases of some other methods to overcome this problem. For this effort, a mobile phase pH well above the pK_a was chosen to aid retention, and the XTerra column was chosen because of its ability to accommodate high pH mobile phases. It was expected that clindamycin and related components would not be partially ionized within this range, which is generally required for a robust separation.

After the initial mobile phase composition and column were chosen, DryLab® chromatography modeling software was used to optimize the separation. Calibration runs consisting of 20- and 60-min gradient ramps were conducted. A mixture containing the peaks of interest was analyzed, along with solutions containing individual impurities and TEC. Entry of the data from the calibration runs into DryLab® permitted us to model the effects of changes in the separation conditions.

The conditions chosen for further investigation and eventual validation (Section 2.2) resulted in the separation depicted in Fig. 2. Authentic supplies of one of the impurities, 4-desoxy-4-α-chloroclindamycin B, were not available to confirm its identity. However, the small peak at 14.4 min was tentatively identified as 4-desoxy based on the relative size of the peak, its order of elution, and its proximity to the clindamycin peak.

A severe negative baseline slope that started with the onset of the gradient was attributed to a lower concentration of buffer in mobile phase B. Because of solubility limitations, the additional buffer required to offset the decline in absorbance could not be added to mobile phase B. The sloping baseline was largely mitigated with the addition of a small quantity of $NaNO_3$, a practice known as "absorbance matching" [15].

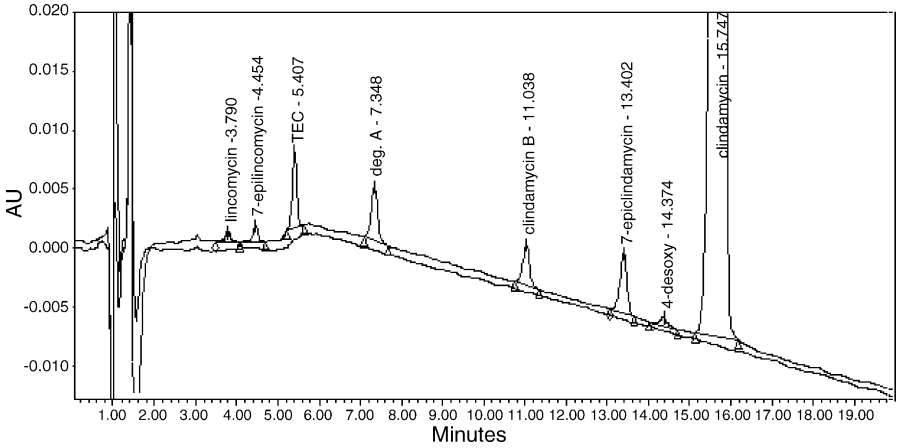

Fig. 2. Expanded chromatography for a mixture of clindamycin and impurities (including TEC, top), and mobile phase A blank (bottom).

Table 3
Linear regression data for clindamycin and minor components

	Clindamycin	Lincomycin	7-Epilincomycin
Concentration range (%)	70–130	0.1–5.0	0.1–5.0
Slope	40177	56143	48793
Intercept	4194.5	26.106	24.512
Correlation coefficient (r)	0.99997	0.99997	0.99998
RMSE (or ESD)	279.94	30.401	19.170
STD error, slope	184.12	249.92	172.56
STD error, intercept	564.66	19.412	12.241
RMS-X (full fit)	0.006964	0.0005415	0.0003930
F_R	N/A	1.40	1.21
	Deg. Prod. A	Clindamycin B	7-Epiclindamycin
Concentration range (%)	0.1–5.0	0.1–5.0	0.1–5.0
Slope	53587	42647	40864
Intercept	−2.5671	−8.2747	−12.494
Correlation coefficient (r)	0.99999	0.99995	0.99998
RMSE (or ESD)	14.738	29.442	14.954
STD error, slope	121.40	249.41	133.83
STD error, intercept	9.4097	18.798	9.5484
RMS-X (full fit)	0.0002750	0.0006904	0.0003660
F_R	1.33	1.06	1.02

3.2. Linearity, LOD, and calculation of relative response factors

Linearity of detector response (peak area) versus concentration was evaluated for clindamycin and five related substances. The regression data for all of the components tested are shown in Table 3. UV relative response factors (F_R) were calculated for each impurity using the following equation:

$$F_R = \frac{S_{imp}}{S_{clindamycin}}$$

where S_{imp} is the slope of the regression line for a given impurity and $S_{clindamycin}$ is the slope of the regression line for clindamycin. All impurity concentrations except degradation product A were corrected for approximate purity. The clindamycin concentration was also corrected for purity. The data shown in Table 2 confirmed the detector response at 214 nm was linear over the ranges tested for all components.

Based on 3× S/N, the LOD was calculated to be 0.03%. Calculations were based on the response observed for the 0.1% 7-epiclindamycin linearity sample. This impurity was chosen because it has the lowest relative response factor of all of the impurities tested.

3.3. Accuracy, precision, and LOQ

Lincomycin was chosen as the sole impurity for accuracy and precision testing for two reasons. First, a well-characterized supply of authentic material was available. Second, the clindamycin drug substance lot used for the experiment contained no measurable amounts of lincomycin. Therefore, no correction was required to account for lincomycin already present in the drug substance. For accuracy and precision testing, lincomycin was considered to be a suitable surrogate for most of the other known impurities because of their structural similarities.

Table 4 contains the data obtained for the accuracy and precision measurements for clindamycin and lincomycin. Acceptable accuracy and precision values at the 0.1% level demonstrated the LOQ was not >0.1%.

3.4. Robustness

Because other methods have been shown to be sensitive to minor pH fluctuations, we believed it important to demonstrate robustness with respect to moderate changes in mobile phase pH. A resolution mixture was constructed containing several components of interest, including TEC. Moderate mobile phase pH changes were shown to have virtually no effect on the chromatography; almost no change in retention times, peak shape, or column efficiency was noted.

With respect to solution stability, <1% change was noted in potency values over 72 h for both ambient and refrigerated solutions. No new impurities at levels equal to or greater than the LOQ were noted in the sample chromatography.

3.5. Assay of tablet samples

Actual tablet samples were assayed using the conditions described in this document. Both multi-tablet potency

Table 4
Accuracy and precision data for clindamycin and lincomycin

Sample no.	Clindamycin added (mg)	Clindamycin recovered (mg)	Recovered (%)
1	596.9	592.5	99.3
2	595.7	591.4	99.3
3	601.7	596.3	99.1
Average (i.e., accuracy)	–	–	99.2
R.S.D. (%)	–	–	0.12
	Lincomycin added (%)	Lincomycin recovered (%)	Recovered (%)
1	0.0907	0.0897	98.9
2	0.0961	0.0899	93.5
3	0.0892	0.0890	99.9
Average (i.e., accuracy)	–	–	97.4
R.S.D. (%)	–	–	3.5

(composite sample) and content uniformity testing was performed. R.S.D.s of <2% were obtained for several content uniformity analyses. This is indicative of both a well-controlled manufacturing process and precise analytical methodology.

4. Conclusion

An HPLC assay for the determination of clindamycin and related impurities in a novel tablet formulation was developed and validated. The assay utilized a previously unreported set of conditions, including a gradient ramp and high-pH mobile phases, to effect the separation without the use of an ion-pair reagent in the mobile phase. A gradient separation was developed to separate TEC (a formulation excipient) as well as a degradation product from other components of interest, and to efficiently elute a wide range of compounds of different polarities (this to accommodate the unknown degradation profile of a developmental formulation). The method exhibited good selectivity and sensitivity. The assay was validated and shown to be appropriate for its intended use, and was used to test actual clindamycin tablet samples.

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