Oxidation study of Clindamycin and its related esters in their Pharmaceutical dosage forms by HPLC and LC-MS.
6.1 Introduction of Clindamycin and its esters

6.1.1 Clindamycin

Clindamycin, a very well known antibiotic that is highly effective against Gram-positive and Gram-negative anaerobic pathogens as well as Gram-positive aerobes. Clindamycin is a white crystalline powder and usually available as its hydrochloride salt. It is soluble in water and other solvents such as methanol, Acetone etc. Clindamycin melts at approximately 141°C-142°C. The empirical formula of Clindamycin is \( \text{C}_{18}\text{H}_{33}\text{N}_2\text{O}_5\text{SCI} \). The molecular weight of Clindamycin is 424.9 [461.4 for HCl salt]. The material should be stored at 2-8°C.

6.1. FI: Chemical structure of Clindamycin

Molecular formula: \( \text{C}_{18}\text{H}_{33}\text{N}_2\text{O}_5\text{SCI} \)
Molecular Weight: 424.9
6.1.2 Clindamycin phosphate

Clindamycin phosphate, a phosphate ester of Clindamycin and very well known highly effective antibiotic. Clindamycin phosphate is a white crystalline powder. It is soluble in water and other solvents such as methanol, Acetone etc. Clindamycin phosphate melts at approximately 114°C. The empirical formula of Clindamycin is $\text{C}_{18}\text{H}_{34}\text{ClN}_2\text{O}_8\text{PS}$. The molecular weight of Clindamycin is 504.96. The IUPAC name is Methyl7-chloro-6,7,8-trideoxy-6-(1-methyl-trans-4-propyl-L-2-pyrrolidine-carboxamido)-1-thio-L-threo-alpha-D-galacto-octopyranoside-2-dihydrogen phosphate. The material should be stored at 2-8°C.

6.1. F2: Chemical structure of Clindamycin phosphate

![Chemical structure of Clindamycin phosphate]

**Molecular formula:** $\text{C}_{18}\text{H}_{34}\text{ClN}_2\text{O}_8\text{PS}$

**Molecular Weight:** 504.96
6.1.3 Clindamycin palmitate:

Clindamycin palmitate, a palmitate ester of Clindamycin and is also a well known, effective antibiotic. Clindamycin palmitate is a white solid. It is also soluble in water and freely soluble in methanol. Clindamycin palmitate melts at approximately 141-143°C. The empirical formula of Clindamycin is C$_{34}$H$_{64}$Cl$_2$N$_2$O$_6$S. The molecular weight of Clindamycin is 699.85. The IUPAC name is Methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-trans-4-propyl-L-2-pyrrolidine-carboxamido)-1-thio-L-threo-alpha-D-galacto-octopyranoside-2-palmitate. The material should be stored at 2-8°C.

6.1.F3: Chemical structure of Clindamycin Palmitate

![Chemical structure of Clindamycin Palmitate](image)

**Molecular formula:** C$_{34}$H$_{64}$Cl$_2$N$_2$O$_6$S

**Molecular Weight:** 699.85
6.2 Pharmacology and Discussion on methods

Clindamycin is an antibiotic, similar to and a derivative of Lincomycin. Clindamycin can be used in topical or systemic treatment. It is effective as an anti-anaerobic antibiotic and anti-protozoal. It is a semi synthetic lincosamide antibiotic that has largely replaced Lincomycin due to an improved side effect profile. Clindamycin inhibits bacterial protein synthesis by binding to bacterial 50S ribosomal subunits. It may be bacteriostatic or bactericidal depending on the organism and drug concentration [143-147].

For the treatment of serious infections caused by susceptible anaerobic bacteria, including Bacteroides spp., Peptostreptococcus, anaerobic streptococci, Clostridium spp., and microaerophilic streptococci. It is useful in polymicrobial infections such as intra-abdominal or pelvic infections, osteomyelitis, diabetic foot ulcers, aspiration pneumonia and dental infections. Can also be used to treat MSSA and respiratory infections caused by S. pneumoniae and S. pyogenes in patients who are intolerant to other indicated antibiotics or who are infected with resistant organism. May be used vaginally to treat vaginosis caused by Gardnerella vaginosa. Clindamycin reduces the toxin producing effects of S. aureus and S. pyogenes and as such, may be particularly useful for treating necrotizing fasciitis. It is widely used topically to treat acne.
Systemic/vaginal Clindamycin inhibits protein synthesis of bacteria by binding to the 50S ribosomal subunits of the bacteria. Specifically, it binds primarily to the 23s RNA subunit. Topical clindamycin reduces free fatty acid concentrations on the skin and suppresses the growth of Propionibacterium acnes (Corynebacterium acnes), an anaerobe found in sebaceous glands and follicles. Rapidly absorbed after oral administration with peak serum concentrations observed after about 45 minutes. Absorption of an oral dose is virtually complete (90%) and the concomitant intake of food does not appreciably modify the serum concentrations; serum levels have been uniform and predictable from person to person and dose to dose [151-156].

This antibiotic is highly effective against Gram-positive and Gram-negative anaerobic pathogens as well as Gram-positive aerobes. Its phosphate and palmitate esters, Clindamycin-2-phosphate and Clindamycin palmitate hydrochloride are produced by chemical modification of Clindamycin. Although these esters are not biologically active, they are rapidly hydrolysed to active clindamycin in-vivo. Clindamycin palmitate is available only as a oral solution for pediatric use and clindamycin is offered in the form of capsules orally. Clindamycin phosphate is administered as a sterile solution, topical solution, cream, gel and vaginal suppositories. The sterile solution is indicated for the treatment of serious infections caused by susceptible
anaerobic bacteria and topical solution is useful for the treatment of acne vulgaries. Clindamycin phosphate is also available as a combination product with benzyl peroxide for topical treatment. The list of available dosage forms of Clindamycin and its esters is detailed in Table 6.2.T1.

A list of HPLC methods combined with UV detection and mass spectrometry are employed for the determination of Clindamycin, Clindamycin phosphate and their related substances in their pharmaceutical dosage forms [157-164]. Couples of HPLC methods are also published for the determination of Clindamycin in human liquids and biological fluids. In United States pharmacopoeia and European pharmacopea methods are reported for the determination of Clindamycin and its esters in pharmaceutical dosages. In all the above methods, the common related impurities are Lincomycin, Lincomycin phosphate, clindamycinB, clindamycinB-2-phosphate, clindamycin-3-phosphate, clindamycin-4-phosphate, epiclindamycin and epilincomycin. Out of these Lincomycin and Lincomycin phosphate are hydrolysis products.

The oxidation study remained unknown till date for pharmaceutical dosage forms of clindamycin and its related esters. Clindamycin and its esters are in soluble form in most of their pharmaceutical dosage forms and hence are very sensitive towards environmental oxidation. In the current study, we present evidence for the oxidative degradation of clindamycin and its related esters in all pharmaceutical dosage forms.
using HPLC and LC-MS. Studies were performed according to prescribed ICH guidelines (forced degradation studies) to evaluate the inherent stability of the drug. Accelerated and long term stability studies were also conducted for the pharmaceutical dosage forms containing clindamycin and its related esters.

6.2. T1: Details of Clindamycin and its esters-Dosage forms

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Generic Name</th>
<th>Dosage</th>
<th>Strength</th>
<th>Route of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleocin</td>
<td>Clindamycin HCl</td>
<td>Solid/Capsules</td>
<td>75mg, 150mg, 300mg</td>
<td>Oral</td>
</tr>
<tr>
<td>Cleocin</td>
<td>Clindamycin Phosphate</td>
<td>Liquid</td>
<td>150mg/mL</td>
<td>Injection (I.V)</td>
</tr>
<tr>
<td>Clindates</td>
<td>Clindamycin Phosphate</td>
<td>Pledgets</td>
<td>10mg/mL</td>
<td>Topical</td>
</tr>
<tr>
<td>Benzaclen</td>
<td>Clindamycin Phosphate and Benzyl peroxide</td>
<td>Gel</td>
<td>1% and 5%</td>
<td>Topical</td>
</tr>
<tr>
<td>Clindesse</td>
<td>Clindamycin Phosphate</td>
<td>Cream</td>
<td>2%</td>
<td>Vaginal</td>
</tr>
<tr>
<td>Evocin</td>
<td>Clindamycin Phosphate</td>
<td>Foam</td>
<td>2%</td>
<td>Topical</td>
</tr>
<tr>
<td>Acanya</td>
<td>Clindamycin Phosphate and Benzyl peroxide</td>
<td>Gel</td>
<td>1.2% and 2.5%</td>
<td>Topical</td>
</tr>
<tr>
<td>Cleocin Ovules</td>
<td>Clindamycin Phosphate</td>
<td>Suppositories</td>
<td>4%</td>
<td>Vaginal</td>
</tr>
<tr>
<td>Cleocin pediatric</td>
<td>Clindamycin Palmitate HCl</td>
<td>Granules</td>
<td>1.5%</td>
<td>Oral</td>
</tr>
</tbody>
</table>
6.3. Materials and Equipment

Samples of Clindamycin and its esters were received from Versapharm Incorporated, Warminster, PA, and USA. In addition, Clindates and Cleocin pediatric were procured from Versapharm Incorporated. Other commercially available drug products listed in the table 6.2.T1 containing Clindamycin and its esters were purchased. HPLC grade Acetonitrile and methanol were purchased from Merck, (Darmstadt, Germany). Analytical reagent grade Potassium phosphate monobasic, Ammonium Acetate, phosphoric acid and acetic acid were purchased from Merck. Highly pure water was prepared with the Millipore Milli-Q Plus water purification system.

The LC system, used for method development, forced degradation studies and for method validation was Waters 2695 binary pump with auto sampler and a 2996 photo diode array detector. The output signal was monitored and processed using empower software on Pentium computer (Digital equipment Co.). LC-MS system (Agilent 1100 series liquid chromatography system coupled with 6400 series triple quadrupole mass spectrometer) was used for the identification of unknown compound formed during forced degradation. Stability Chambers for 25°C/60%RH (long term conditions) TESCOR INC., USA and for 40°C/75%RH (Accelerated conditions) FORMA Scientific, USA were used for studies.
6.4 Standard and Samples Preparation

6.4.1 Clindamycin

A working test solution of Clindamycin (2.0 mg/ml) was prepared by dissolving appropriate amount in the water as diluent.

Five capsules were weighed and the powder emptied from the capsules into a clean dry mortar and mixed well using a pestle. Powder equivalent to 100 mg drug was transferred into a 50 mL volumetric flask; 20 mL of water was added. The flask was attached to a rotary shaker and shaken for 5 min to disperse the powder completely. The mixture was sonicated for 5 min and then diluted to the volume with diluent to make a solution containing 2.0 mg/mL. This solution was centrifuged at 3000 RPM for 5 min. The solution was filtered through 0.45μ nylon 66-membrane filter.

6.4.2 Clindamycin Phosphate

A working test solution of Clindamycin phosphate (2.0 mg/ml) was prepared by dissolving appropriate amount in the diluent.

During the preparation of Clindets test solution, the pledgets were squeezed into a fresh bottle and 2g of sample equivalent 20mg of Clindamycin was weighed in to a 10mL volumetric flask and diluted to volume with diluent. The solution was filtered through 0.45μ nylon 66-membrane filter. This same procedure was also followed for the sample preparation of Evoclin foam.
In the preparation of gel test solution [Benzaclin, Acanya, Veltin], 5g of gel was taken into a 50mL screw capped test tube and added 25mL of 1% Calcium chloride was added and vortex well to disperse the gel and then diluted to 50mL with diluent and mixed well. The solution was filtered through 0.45μ nylon 66-membrane filter.

In the case of preparation of cream test solution [Clindesse], 5g of cream was taken into a 50mL screw capped test tube and added 25mL of diluent and was warmed in a water bath at 50°C for 5min to disperse the cream and vortex well at hot condition. Diluted to 50mL with diluent and mixed well. The solution was filtered through 0.45μ nylon 66-membrane filter.

6.4.3 Clindamycin Palmitate

A working test solution of Clindamycin Palmitate (20 mg/mL) was prepared by dissolving appropriate amount in the diluent.

Cleocin pediatric, the granules in the bottle was reconstituted with 75mL of water and shaked well to dissolve. For preparation of the working test solution, 5g of the test sample solution diluted to 10mL with diluent.
6.5. Specificity and generation of stress samples

Pharmaceutical products are especially sensitive to variety of environmental factors such as temperature, humidity, light and oxidation. Acid and base are typically employed to accelerate the evaluation of hydrolytic stability of a chemical entity. Nevertheless, non-hydrolytic degradation may occur and sometimes can become the dominant degradation process.

All the stress studies on Clindamycin and its esters were well known in the literature. The oxidation study remained unknown or relatively less known till date for pharmaceutical dosage forms of clindamycin and its related esters. Moreover, Clindamycin and its esters are in, soluble form in most of their pharmaceutical dosage forms and hence are very sensitive towards environmental oxidation. In the current study, we present evidence for the detailed oxidative degradation of clindamycin and its related esters in all pharmaceutical dosage forms using HPLC and LC-MS. Studies were performed according to prescribed ICH guidelines (forced degradation studies) to evaluate the inherent stability of the drug. Accelerated and long term stability studies were also conducted for the pharmaceutical dosage forms containing clindamycin and its related esters.
Oxidative stress:

Drug was exposed at room temperature for 4Hrs, 8Hrs, 12Hrs and 24Hrs in 1% \( \text{H}_2\text{O}_2 \) (v/v) and 3% \( \text{H}_2\text{O}_2 \) (v/v).

The above stress conditions were also applied on all the pharmaceutical dosage forms of Clindamycin & its esters to check the applicability of the developed method for the drug product. The peak purity of the Clindamycin and its esters, stressed samples were checked by using a Waters 2996 photo diode array detector (PDA).

6.6 Method development

Enormous literature was available for the separation and quantification of impurities by HPLC. Hence method development was initiated and optimized using those methods. The methods were finalized by using oxidation degradation samples and placebo samples used for the preparation of pharmaceutical dosage forms.

Clindamycin and its esters are low responsive in UV. All samples were prepared in diluent at a concentration of 10ppm and injected individually. They were scanned through the photo diode array (PDA) detector. UV spectrums of Clindamycin and also its esters have shown common UV maxima at around 205nm. The base line noise is more at that wavelength as most used solvents have UV cut-off around 200nm. Hence detection at 215 nm was selected for the optimization of method development.
6.6.1: Typical UV spectrums

6.6.1. F1: Clindamycin

6.6.1. F2: Clindamycin Phosphate

6.6.1. F3: Clindamycin Palmitate

300
6.7 Optimized Chromatographic Conditions

6.7.1 Clindamycin and Clindamycin Phosphate

Column: Waters Symmetry C-18, 250mm x 4.6mm, 5μ Particle size

Mobile phase-A: Buffer

Mobile phase-B: Water: Acetonitrile: 10:90 (v/v)

Buffer: 0.02M Potassium dihydrogen phosphate, pH adjusted to 5.0 with NaOH.

Elution: Gradient

Flow rate: 1.0 mL/min

Column temperature: 30°C

Wavelength of detection: 215 nm

Injection volume: 20 μL

Run time: 45 min

Diluent: Water: Acetonitrile (8:2, v/v)

Gradient program:

<table>
<thead>
<tr>
<th>Time in min</th>
<th>0</th>
<th>5</th>
<th>35</th>
<th>40</th>
<th>41.0</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase B</td>
<td>10%</td>
<td>10%</td>
<td>40%</td>
<td>40%</td>
<td>10%</td>
<td>10%</td>
</tr>
</tbody>
</table>
6.7.2 Clindamycin Palmitate HCl

Column: Waters Symmetry C-18, 250mm x 4.6mm, 5μ Particle size

Mobile phase: Buffer: Methanol 5:95 (v/v)

Buffer: 3% Ammonium Acetate in water.

Elution: Isocratic

Flow rate: 1.0 mL/min

Column temperature: 30 ± 2°C

Wavelength of detection: 215 nm

Injection volume: 20 μL/50 μL

Run time: 50 min

Diluent: Water: Methanol (2:8, v/v)

Retention Time 25min

6.8 Specimen Chromatograms

6.8. F1: Clindamycin -Test Sample
6.8. F2: Clindamycin Phosphate-Test Sample

6.8. F3: Clindamycin Palmitate-Test Sample
6.9 Forced/stress degradation

As discussed in section 6.5, extensive oxidation studies were only conducted and studied on Clindamycin and its esters. HPLC and LC-MS studies of the formed impurities under oxidative stress conditions are detailed below.

6.9.1 Oxidation

As oxidation is one of the major degradation processes employed to evaluate the stability of the chemical entity. Clindamycin and its esters were exposed with 0.5% hydrogen peroxide (mild environment) and 3% hydrogen peroxide (elevated environment) at room temperature for 24 hours. Clindamycin and its esters degraded significantly at various altitudes when exposed to both mild and elevated oxidative conditions [6.9.1.T1]. It was observed that, Clindamycin, Clindamycin phosphate and Clindamycin palmitate oxidized in the sliding order respectively.

Clindamycin oxidized up to 10% when it was exposed for 2Hrs and it was totally degraded after 24 Hrs. Where as Clindamycin phosphate oxidized up to 5% when it was exposed for 2Hrs and up to 70% after 24Hrs but when it comes to Clindamycin palmitate the oxidation intensification is deliberate. Clindamycin palmitate degraded to 1%, 3% and 10% after 2Hrs, 8Hrs and 24Hrs respectively. These variations in the intensity of oxidation can be attributed towards the bulky functional group [phosphate, palmitate] present in the Clindamycin esters than
Clindamycin alone. When compared to phosphate functional group palmitate is much more massive and hence the altitude of oxidation is more in Clindamycin phosphate than Clindamycin palmitate.

6.9.1. T1: Oxidation

<table>
<thead>
<tr>
<th>Time</th>
<th>% oxidation in 0.5% H₂O₂</th>
<th>% oxidation in 3% H₂O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2Hrs</td>
<td>8Hrs</td>
</tr>
<tr>
<td>Clindamycin HCl</td>
<td>5%</td>
<td>39%</td>
</tr>
<tr>
<td>Clindamycin Phosphate</td>
<td>2%</td>
<td>11%</td>
</tr>
<tr>
<td>Clindamycin Palmitate</td>
<td>0.5%</td>
<td>1.8%</td>
</tr>
</tbody>
</table>

6.9.2 Typical Chromatograms – Oxidation

6.9.2. F1: Clindamycin-0.5%H₂O₂ 8Hrs
6.9.2. F2: Clindamycin-Peak Purity report

<table>
<thead>
<tr>
<th>Purity Angle</th>
<th>Purity Threshold</th>
<th>Purity Flag</th>
<th>Peak Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.321</td>
<td>0.423</td>
<td>No</td>
<td>Pass</td>
</tr>
</tbody>
</table>

6.9.2. F3: Clindamycin Phosphate -0.5%H$_2$O$_2$ 8Hrs
6.9.2. F4: Clindamycin Phosphate – Peak Purity

<table>
<thead>
<tr>
<th>Purity Alert</th>
<th>Purity Threshold</th>
<th>Purity Flag</th>
<th>Peak Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.239</td>
<td>0.275</td>
<td>No</td>
<td>Pass</td>
</tr>
</tbody>
</table>

6.9.2. F5: Clindamycin Palmitate - 3% H₂O₂ 24Hrs
6.9.2. F6: Clindamycin Palmitate – Peak Purity

Peak purity test results derived from PDA detector confirmed that, the peaks of Clindamycin & its esters are homogeneous and pure in all the analyzed stress samples i.e. no hidden coelution is there along with principal peaks.

6.10 Identification of Degradation Impurities

A major impurity found during the oxidative stress in all the samples of Clindamycin & its esters. The relative retention times of the impurities formed are ~0.72, ~0.80 and ~0.45 respectively in Clindamycin, Clindamycin phosphate and Clindamycin palmitate (6.9.2). Interestingly, the same impurity also enhanced in all the pharmaceutical dosage forms containing Clindamycin and its esters. A detailed study on

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the stability samples of the available dosage forms was performed and reported in the section 6.10.T1. A mounting trend of oxidation impurity noticed in all dosage forms of Clindamycin and its dosage forms. This degradation in the pharmaceutical dosage forms is due to environmental oxidation. The quantum of impurity formed is more in the liquid dosages when compared to semisolids and solid dosage forms. This variation could be attributed to the solubility status in the respective dosage forms. An attempt was made to identify the formed impurities using UV-spectrum and LC-MS.

**6.10. T1: Stability Report-% Oxidation Impurity**

<table>
<thead>
<tr>
<th>Generic Name</th>
<th>Dosage</th>
<th>Initial</th>
<th>3M/40°C/75%</th>
<th>6M/25°C/60%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clindamycin HCl</td>
<td>Solid/Capsules</td>
<td>0.51%</td>
<td>0.65%</td>
<td>0.55%</td>
</tr>
<tr>
<td>Clindamycin Phosphate</td>
<td>Liquid</td>
<td>0.25%</td>
<td>1.88%</td>
<td>1.21%</td>
</tr>
<tr>
<td>Clindamycin Phosphate</td>
<td>Pledgets</td>
<td>0.45%</td>
<td>1.81%</td>
<td>1.01%</td>
</tr>
<tr>
<td>Clindamycin Phosphate</td>
<td>Cream</td>
<td>0.55%</td>
<td>1.55%</td>
<td>1.18%</td>
</tr>
<tr>
<td>Clindamycin Phosphate</td>
<td>Foam</td>
<td>0.45%</td>
<td>1.57%</td>
<td>0.95%</td>
</tr>
<tr>
<td>Clindamycin Phosphate</td>
<td>Suppositories</td>
<td>0.58%</td>
<td>1.52%</td>
<td>0.85%</td>
</tr>
<tr>
<td>Clindamycin Palmitate HCl</td>
<td>Granules</td>
<td>0.21%</td>
<td>1.85%</td>
<td>0.52%</td>
</tr>
</tbody>
</table>

*3M/40°C/75%: Accelerated conditions for 3 months.
6M/25°C/60%: Long term conditions for 6 months.
### 6.10.1 Mass Spectrometry-Optimized Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Column</strong></td>
<td>Waters Symmetry C-18, 250mm x 4.6mm, 5μm Particle size</td>
</tr>
<tr>
<td><strong>Mobile phase:</strong></td>
<td>Ammonium Acetate: Acetonitrile: Acetic acid 60:40:1(v/v)</td>
</tr>
<tr>
<td>(Clindamycin and Clindamycin Phosphate)</td>
<td></td>
</tr>
<tr>
<td><strong>Mobile phase:</strong></td>
<td>Ammonium Acetate: Methanol (5:95 v/v)</td>
</tr>
<tr>
<td>(Clindamycin Palmitate)</td>
<td></td>
</tr>
<tr>
<td><strong>Elution:</strong></td>
<td>Isocratic</td>
</tr>
<tr>
<td><strong>Flow rate:</strong></td>
<td>1.0 mL/min</td>
</tr>
<tr>
<td><strong>Wavelength of detection:</strong></td>
<td>215 nm</td>
</tr>
<tr>
<td><strong>Injection volume:</strong></td>
<td>10 μL</td>
</tr>
<tr>
<td><strong>Capillary Voltage:</strong></td>
<td>3.5 (kV)</td>
</tr>
<tr>
<td><strong>Cone Voltage:</strong></td>
<td>25.0 (v)</td>
</tr>
<tr>
<td><strong>Extractor:</strong></td>
<td>2.00 (v)</td>
</tr>
<tr>
<td><strong>Source Temperature:</strong></td>
<td>150° C</td>
</tr>
<tr>
<td><strong>Dissolvation Temperature:</strong></td>
<td>375° C</td>
</tr>
<tr>
<td><strong>Gas flow:</strong></td>
<td>500 l/Hr</td>
</tr>
</tbody>
</table>
6.10.2 UV Spectrums-Comparison

6.10.2. F1: Clindamycin and Oxidized impurity

6.10.2. F2: Clindamycin Phosphate and Oxidized impurity

6.10.2. F3: Clindamycin Palmitate and Oxidized impurity
6.10.3 Mass spectrums of Degradation Impurities

6.10.3. F1: Clindamycin [Impurity @ RRT~0.72]

6.10.3. F2: Clindamycin Phosphate [Impurity @ RRT~0.80]

6.10.3. F3 Clindamycin Palmitate [Impurity @ RRT~0.45]
6.10.4 Structure Elucidation.

The contrast in UV spectrums of the principal peaks and the degradation impurities were presented in section 6.10.2. A clear bump at 230nm is noticed in all the oxidized impurities of Clindamycin and its esters when compared to their respective principal compounds. This secondary $\lambda_{\text{max}}$ confirms the accumulation of $\pi$ electrons or ring formation during the oxidation process.

The mass spectrums in the positive electron spray ionization (ESI) mode for the impurities formed were shown in section 6.10.3. The m/z values obtained for degradation product in Clindamycin, Clindamycin phosphate and Clindamycin palmitate are 441.4, 521.4 and 679.5 respectively. The molecular mass [M+] for all the three impurities is 16 units more than the molecular mass of respective parent molecule [425,505 and 663.5 respectively]. This indicates there is degradation on the Clindamycin moiety itself where there is addition of 16 units of molecular mass. As the stress applied is oxidative and hence it is suggested that, oxygen addition [whose molecular mass is 16] might have happened on the Clindamycin moiety. When the Clindamycin moiety is further examined, the possible addition of oxygen to the moiety is on to sulfur atom or on to nitrogen atom by forming sulfoxide or N-oxide respectively. The formation of sulfoxide is predominant as bulky groups surround the nitrogen atom where as sulfur atom is free for
addition. This investigation is further supported by the LC-MS/MS analysis of the formed impurity in Clindamycin phosphate. The MS/MS results show same fragmentation pattern as that of Clindamycin phosphate and it is further explained in the section 6.10.4.F2 and 6.10.4.F3. The possible mechanism for the formation of sulfoxide impurity is given below in section 6.10.4.F1.

6.10.4. F1: Suggested Mechanism of formation

![Chemical diagrams illustrating the mechanism of formation of sulfoxide impurities in Clindamycin and Clindamycin phosphate.]

\[ R = H, \text{Clindamycin} \]
\[ R = \text{PO}_3^-, \text{Clindamycin phosphate} \]
\[ R = \text{OC}_{16}H_{31}, \text{Clindamycin palmitate} \]

\[ R = H, \text{Clindamycin sulfoxide} \]
\[ R = \text{PO}_3^-, \text{Clindamycin phosphate sulfoxide} \]
\[ R = \text{OC}_{16}H_{31}, \text{Clindamycin palmitate sulfoxide} \]
6.10.4. F2: LC-MS/MS for Clindamycin Phosphate

Counts vs. Acquisition Time (min)

Counts vs. Mass-to-Charge (m/z)

User Spectra

Fragmenter Voltage | Collision Energy | Isolation Mode |
-------------------|-----------------|----------------|
135                | 25              | Es             |

Peak List

<table>
<thead>
<tr>
<th>m/z</th>
<th>Abund</th>
</tr>
</thead>
<tbody>
<tr>
<td>125.9</td>
<td>910</td>
</tr>
<tr>
<td>456.8</td>
<td>1472</td>
</tr>
<tr>
<td>520.9</td>
<td>5721</td>
</tr>
</tbody>
</table>

315
6.10.4. F3: Fragment Pattern-MS/MS

\[
\begin{align*}
\text{M.Wt.} & \quad -457.86 \\
\text{M.Wt.} & \quad -456.86 \\
\text{M.Wt.} & \quad -126.22
\end{align*}
\]
6.11 Conclusion

In this chapter the oxidation of well-known antibiotics, Clindamycin and its esters were studied to the extent. To my present knowledge these studies were neither performed nor published. This study will be of great help in the pharmaceutical development or improvement of drug products containing these critical class of therapeutic agents.