Validated stability indicating analytical method for the
determination of clindamycin phosphate and adapalene in topical
formulation

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ABSTRACT
A reversed phase liquid chromatography (RPLC) method has been devel-oped for the separation of clindamycin phosphate and adapalene in topical
formulation. The method used a Hypersil BDS, 5µm, 250 × 4.6 mm i.d.
column maintained at 35°C. The mobile phase comprises a) 0.1% OPA (ortho phosphoric acid) and b) THF (tetrahydrofuran): ACN (acetonitrile) at the
ratio of 35:65 at a flow rate of 1.0 mL/min. UV detection is performed at 210
nm. The method showed good ruggedness, robustness, linearity and re-
peatability. It is also suitable for formulation.
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INTRODUCTION
Clindamycin is an antibiotic effective against Gram positive aerobes and both Gram negative and Gram positive anaerobic pathogens. It is synthesized by chemi-
cal modification of lincomycin[1], an antibiotic produced by microbial fermentation, to increase biological activ-
ity. Clindamycin HCl is supplied for oral administration in capsules. Further improvement in pharmaceutical
properties of clindamycin obtained by chemical modific-
tion to obtained the esters, clindamycin phosphate or
clindamycin palmitate[2]. Chemically clindamycin is
Methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-trans-4-propyl–L-2-pyrrolidinecarboxamido)-1-thio-L-threo-
α-D-galacto octopyranoside-2(dihydrogen phosphate)
as shown in Figure 1.

Figure 1: Structure of clindamycin phosphate
Several methods have been reported for the deter-
mination of clindamycin in bulk drug and dosage forms.
Microbiologically[3-4] and spectrophotometric[5] meth-

ods described for assay of clindamycin are non-specific and less accurate. Gas liquid chromatography (GLC) method requires elaborate extraction and derivatisation steps\cite{6,7}. Zhou hue,zhiguo zheng separate and characterize clindamycin and related impurities in bulk drug by HPLC Electrospray tandem mass spectroscopy\cite{8}. The current united state pharmacopoeia (USP) method for determining clindamycin HCl also utilized a RPLC method with refractive index detection and comparable to other method described in the literature\cite{8,9}. Due to poor sensitivity associated with refractive index detection, UV detection is preferable.

Adapalene is third generation synthetic retinoid used in the treatment of acne. It is highly lipophilic compound, derived from naphthoic acid\cite{10}, and chemically designated as 6-[3-(1-adamantyl)-4 methoxy phenyl] -2-naphthoic acid\cite{11}. (Figure 2) In comparison with tretinoin, a first generation retinoid, adapalene presents similar\cite{12} or even better, efficacy\cite{13} and improved tolerability which makes it clinically advantageous. Also adapalene is more stable to light and oxidation by benzoyl peroxide than tretinoin. Adapalene systemic absorption is minimal, and no evidence of teratogenicity has been reported.

![Figure 2: Structure of adapalene.](image)

Method for the determination of adapalene using solid phase extraction and gradients high performance liquid chromatography (HPLC) with UV and fluorescence detection were published\cite{14,15}. However, these methods were intended for the analysis of biological samples and require sample pretreatment. In the present study, the quality control was focused, so the method was suitable for the routine analysis of both raw material and pharmaceutical product. Besides, it possessed a stability indicating nature, which meant that it was capable of detecting and quantifying adapalene in stressed sample, generated by forced decomposition studies\cite{16,17}, in accordance with the international conference on harmonization (ICH) guidelines\cite{17,18}, the U.S. food and drug administration (FDA)\cite{19,20} and the USP\cite{21}. Therefore, this report present a strategy which allows for the quantification of adapalene in topical gel formulation trough a simple, fast and isocratic HPLC diode array detection (HPLC-DAD) method with a short retention time, reaching excellent peaks symmetry.

**EXPERIMENTAL**

**Reagents and samples**

The reagents OPA, ACN, THF and methanol was from Merck and of HPLC grade. Milli-Q-water was used for dilution. The active pharmaceutical ingredients i.e. the clindamycin phosphate and Adapalene were gifted by Glenmark pharmaceutical, Nashik. The sample (clinnmiskin gel formulation contain clindamycin phosphate equivalent to clindamycin 1% and adapalene 0.1%) was obtained from market.

The mobile phase a) 0.1% OPA was prepared by mixing 1 mL of OPA in 1000 mL of milli-Q-water filtered this through 0.45μm membrane filter. The other b) mobile phase was prepared by mixing ACN: THF in the concentration of 65:35. The diluents were methanol and THF.

**LC apparatus and operating conditions**

The Waters LC system consisted of a syringe pump and autosampler set to inject 50 μL, and variable UV detector set at 210 nm. The column was Hypersil BDS, 5μm, 250 × 4.6 mm i. d. The column temperature was maintained at 35°C by means of column oven temperature.

**Analytical procedure**

The reference standard solution of clindamycin phosphate and adapalene was prepared by individually preparing the stock solution of clindamycin phosphate i.e standard stock solution A and for adapalene i.e. standard stock solution B.
Standard stock solution A

Weighed 50 mg of clindamycin phosphate was taken in a 50 mL of volumetric flask, mixed with 30 mL of methanol and sonicated to dissolve and made up the volume up to the mark with methanol.

Standard stock solution B

Weighed 25 mg of adapalene was taken in a 50 mL of volumetric flask, mixed with 20 mL of THF and sonicated to dissolve and made up the volume up to the mark with methanol.

Reference standard solution

Accurately measured 10 mL of standard stock solution A and 2 mL of standard stock solution B was transferred in a 50 mL of volumetric flask and made up the volume up to the mark with methanol.

Marketed sample solution

Accurately weighed 2 g of sample (equivalent to 20 mg of clindamycin phosphate and 2 mg of adapalene) was taken into a 100 mL of volumetric flask and dissolved with 30 mL of THF and sonicated for 15 min., then mixed with 40 mL of methanol and sonicated for 15 min., then made up the volume up to the mark with methanol. Filtered through 0.45 μ nylon filter and injected.

RESULT AND DISCUSSION

Development of chromatographic method

The proposed study was an attempt to develop and validate RP-HPLC method for the determination of clindamycin phosphate and adapalene from reference standard and marketed sample.

During the development of HPLC method, mobile phases were ACN, ammonia buffer, OPA, and THF in different ratios. Mobile phase selection was based on peak parameters i.e. height, asymmetry, tailing, baseline drift, run time, ease of preparation of mobile phase. Keeping all these requirements in consideration, proposed chromatographic condition was found appropriate for quantitative determination.

The chromatographic columns used was Hypersil, BDS, 5μ, 250 × 4.6 mm i.d. and Inertsil, ODS, 5μ, 250 × 4.6 mm i.d. The steps for the method development are as shown in TABLE 1. The chromatogram for reference standard solution as shown in Figure 3.

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Stationary phase</th>
<th>Mobile phase</th>
<th>Retention time (RT) in min</th>
<th>Symmetry (tailing factor)</th>
<th>Theoretical plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Inertsil, ODS, 5μ, 250 × 4.6 mm i.d.</td>
<td>ACN : ammonia buffer (10:90), pH 3.9</td>
<td>13.6</td>
<td>2.28</td>
<td>4754</td>
</tr>
<tr>
<td>2.</td>
<td>Inertsil, ODS, 5μ, 250 × 4.6 mm i.d.</td>
<td>ACN : ammonia buffer (30:70)</td>
<td>7.5</td>
<td>2.14</td>
<td>5213</td>
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<tr>
<td>3.</td>
<td>Hypersil, BDS, 5μ, 250 × 4.6 mm</td>
<td>0.1% OPA and ACN : THF (75:25)</td>
<td>7.28</td>
<td>1.13</td>
<td>10236</td>
</tr>
<tr>
<td>4.</td>
<td>Hypersil, BDS, 5μ, 250 × 4.6 mm</td>
<td>0.1% OPA and ACN : THF (65:35)</td>
<td>4.49</td>
<td>1.31</td>
<td>10580</td>
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</table>

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Stationary phase</th>
<th>Mobile phase</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>Inertsil, ODS, 5μ, 250 × 4.6 mm i.d.</td>
<td>ACN : ammonia buffer (10:90), pH 3.9</td>
<td>Peak was not eluted.</td>
<td>Peak was not eluted</td>
<td>63589</td>
</tr>
<tr>
<td>2.</td>
<td>Inertsil, ODS, 5μ, 250 × 4.6 mm i.d.</td>
<td>ACN : ammonia buffer (30:70)</td>
<td>Peak was not eluted.</td>
<td>Peak was not eluted</td>
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<tr>
<td>3.</td>
<td>Hypersil, BDS, 5μ, 250 × 4.6 mm</td>
<td>0.1% OPA and ACN : THF (75:25)</td>
<td>22.42</td>
<td>1.24</td>
<td>100476</td>
</tr>
<tr>
<td>4.</td>
<td>Hypersil, BDS, 5μ, 250 × 4.6 mm</td>
<td>0.1% OPA and ACN : THF (65:35)</td>
<td>19.18</td>
<td>1.16</td>
<td>110222</td>
</tr>
</tbody>
</table>
In the reference standard solution, the number of theoretical plates for clindamycin phosphate and adapalene was 10580 and 110222 respectively. The % RSD was not more than 2% for both the drugs. The column oven temperature was 35°C. The mobile phases selected were in gradient mode i.e. a) 0.1% OPA and b) ACN: THF in the ratio of 65:35. The mobile phase flow was 1 mL/min. 50μL sample (200μg/mL clindamycin phosphate and 20 μg/mL adapalene) were injected.

The standard solution was run for sufficient period of time at a wavelength 210 nm under the following chromatographic condition as shown in TABLE 2.

After the development of standard solution, the same chromatographic conditions were applied to the marketed sample solution. The chromatogram of sample solution as shown in Figure 4.

**METHOD VALIDATION**

**Linearity**

The calibration curve obtained by replicate analysis of a series of analyte concentration corresponding to 50%, 75%, 100%, 125%, 150%. Injected mass was subjected to linear regression analysis. For clindamycin phosphate \( Y=14772 x + 102447 \), correlation coefficient \( r = 0.9998 \) and for adapalene \( Y= 275009x + 485738 \), correlation coefficient \( r= 0.9991 \) where \( Y \) is the peak area, \( x \) is the mass (μg) injected. The acceptance criteria are that the correlation coefficient should not less than 0.999.

**System precision**

Six reference standard solutions were prepared and analyzed by proposed method. % RSD for clindamycin phosphate and adapalene was 0.3 and 0.5 respectively. The % RSD value indicated that the method has an acceptable level of precision. (Acceptance criteria: % RSD should not be more than 2%).

**Method precision**

Six samples of marketed sample were prepared and analyzed by proposed method. % RSD for clindamycin phosphate and adapalene was 0.9 and 0.7 respectively.

The % RSD value indicated that the method has an acceptable level of precision. (Acceptance criteria: % RSD should not be more than 2%).

**Recovery study / Accuracy study**

The recovery study was performed by preparing samples at three levels i.e. (50%, 100%, and 150%) in triplicate. The samples were prepared and analyzed as per proposed method. The percent recovery for clindamycin phosphate was in the range of 98.9 –
100.6% and for adapalene was 101.4-101.8%.

Result indicated that the method has an acceptable level of accuracy. (Acceptance criteria: recovery should be in the range of 97.0%-103.0%).

**Robustness**

The robustness was determined by injecting six reference standard solutions at each different condition with respect to control condition. Robustness of the method was checked by varying the instrumental conditions such as flow rate (±0.2 mL), organic content in mobile phase ratio (±10%), and wavelength of detection (± 5 nm), column oven temperature (± 5°C) and change in pH of buffer (± 0.2%). Six replicate standard solutions were injected in each condition and calculated the system suitability parameters.

All the parameters of system suitability were passed. The % RSD was not more than 2%. The theoretical plates, tailing factor were within the range. (Acceptance criteria: over all % RSD should not be more than 2%. The theoretical plate should be more than 2000 and tailing factor should not more than 2). The method was found to be robust and no significant changes were observed.

**Ruggedness**

Ruggness of the method was verified by analyzing the six duplicate injections of marketed sample by two different analyst using different instruments on different days. The system suitability parameters were checked. The % RSD of clindamycin phosphate was by analyst 1 was 0.9% and by analyst 2 was 0.7. The % RSD of adapalene was by analyst 1 was 0.7% and by analyst 2 was 0.8. (Acceptance criteria: overall % RSD should not more than 2%). The results were found to be within the limits thus confirming that the method is rugged.

**Stress degradation study**

A stress degradation study was carried out according to the ICH guidelines.

**Hydrolytic degradation**

2 g of marketed samples were treated with 0.2 N HCl, 0.2 N NaOH for one hour individually. Solution of these samples were prepared as per test method, and followed by analysis as per the proposed method. The clindamycin phosphate was degraded by acid was 63.9%, by alkali was 65.9%. The adapalene was not degraded by acid was not occur but by alkali was 3.8%.

**Oxidative degradation**

2 g of marketed sample was treated with 6 % H2O2 for one hour. Solution of these samples was prepared as per test method, and followed by analysis as per the proposed method. The clindamycin phosphate was degraded up to 8.7 and adapalene was not degraded.

**Thermal degradation**

2 g of marketed sample was subjected to thermal degradation at 105°C for one hour. Solution of this sample was prepared as per test method, and followed by analysis as per the proposed method. No any degradation was occurring on clindamycin phosphate and adapalene.

**Photolytic degradation**

Photolytic degradation study was carried out by exposing the 2 g of marketed sample to UV light for 7 days in the UV cabinet. UV energy not less than 200 W/m2, solution of this sample was prepared as per test method, and followed by analysis as per the proposed method. No any degradation was occurring on clindamycin phosphate and adapalene.

**Humidity degradation**

Humidity degradation study was carried out by exposing the 2 g of marketed sample to 90% RH for 7 days. Solution of these samples was prepared as per test method, and followed by analysis as per the proposed method. No any degradation was occurring on clindamycin phosphate and adapalene.

**Result of stress studies**

The clindamycin phosphate was degraded by acid, alkali and peroxide degradation, but not by heat, light and humidity. The adapalene was degraded by alkali hydrolysis but not by any other degradation. Using the peak purity test, the purity of clindamycin phosphate and adapalene peak was checked at every stage of above mentioned studies.

**CONCLUSION**

A simple, easy and cost-effective stability indicat-
ing HPLC method for the determination of clindamycin phosphate and adapalene in reference standard solution and marketed sample was developed using 0.1% OPA and ACN: THF in the ratio of 65:35 as the mobile phase and column was Hypersil, BDS, 5μ, 250 × 4.6 mm i.d.

The method was thoroughly validated and was found to be accurate, precise, linear and robust, rugged and stable. The clindamycin phosphate shows retention time at 4.9 min and adapalene at 18.9 min. The number of theoretical plates for clindamycin phosphate was 10580 and for adapalene were 110222. The % RSD was less than 2% for the entire study. To conclude, the present developed and validated HPLC method appears to be very sensitive, selective, precise, accurate, less time consuming, reproducible and thus suitable for routine analysis for the estimation of clindamycin phosphate and adapalene.

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REFERENCES