HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ADAPALENE IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT
A validated reverse phase HPLC method has been developed for the estimation of Adapalene in Topical Cream. The Chromatographic separation was carried out on Phenomenex C\textsubscript{18} (250 X 4.6 mm, 5 \( \mu \)m) column and Tetra Hydrofuran: Acetonitrile: 0.1\% Acetic acid in water in the ratio of 20:40:40\% v/v was used as mobile phase at the flow rate of 1.2 ml/min with PDA detection at 270 nm. The retention time of Adapalene were found to be 10.44 minutes. Linearity dynamic range was of 10- 30 \( \mu \)g/ml for Adapalene. The developed HPLC method was validated by determining its sensitivity, selectivity, linearity, accuracy and precision. The accuracy of the method was assessed by percentage recovery studies at three different levels at 50\%, 100\% and 150\% of its working concentration. The percentage recovery of the drugs in the developed method was found to be in the ranges of from 98.2\% - 101.7\%, that indicates the good accuracy of the method. This developed method can be used for the routine analysis for the estimation of Adapalene in bulk and Pharmaceutical formulations.

KEY WORDS
Adapalene, RP-HPLC Method and Method validation.

INTRODUCTION
Adapalene is chemically 6-\{3-(adamantan-1-yl)-4-methoxyphenyl\} naphthalene-2-carboxylic acid, which is categorized under Dermatologic agents. Chemical structure of Adapalene is shown in Figure No.1. Adapalene is a third-generation topical retinoid primarily used in the treatment of mild-moderate acne and is also used to treat keratosis pilaris as well as other skin conditions. Adapalene in small concentrations is a moderator of cellular
differentiation, keratinization, and inflammatory processes. It has both exfoliating and anti-inflammatory effects. The exact mode of action of adapalene is unknown. Adapale alone (or) in combined formulation with other drugs is reported to be estimated by HPLC and UV/VIS Spectrophotometric methods. Literature review revealed that several methods have been reported for the quantification of Adapalene individually. The present work describes a new, simple, rapid, accurate and precise RP-HPLC method developed and validated for the estimation of Adapalene simultaneously.

**MATERIALS AND METHOD**

Chromatographic separation was carried out on Shimadzu Prominence liquid chromatographic system equipped with quaternary pump, PDA detector and auto injector. LC solution software (Version 1.23) was used for the entire processing and data collection. All chemicals used were analytical grade and the solvents which are used in the mobile phase were HPLC grade.

**Preparation of Diluent**

Diluent was prepared by mixing the solvents like Tetra Hydrofuran, Acetonitrile and Water in the ratio of 20: 40: 40% v/v respectively. These diluents which was then used in the preparation of standard and sample solution.

**Preparation of standard solution**

Standard solution of Adapalene was prepared by dissolving 20 mg of Adapalene WS in a 100 ml volumetric flask containing 20 ml of Tetra Hydrofuran was added. The content of the flask was sonicated for 10 minutes and the volume was then made up to 100 ml with Tetra Hydrofuran. The resulting solution was further diluted with diluent to get the concentration of 20 µg/ml of Adapalene.

**Preparation of sample solution**

Accurately weighed quantity of sample equivalent to 20 mg of Adapalene was taken in a 100 ml volumetric flask and 20 ml of Tetra Hydrofuran was added. The content of the flask was sonicated for 10 minutes and the volume was then made up to 100 ml with Tetra Hydrofuran. The resulting solution was filtered through whatman filter paper and 5 ml of the filtrate was diluted to 50 ml with diluent.

**Method development and validation**

The RP HPLC procedure was optimized with a view to develop an effective method for the estimation of Adapalene in Pharmaceutical dosage forms. Preliminary tests were performed in order to select the adequate and optimum chromatographic condition. A Phenomenex C18 column was used as a stationary phase and the separation was achieved by using mobile phase consisting of Tetra Hydrofuran: Acetonitrile: 0.1% Acetic acid in water in the ratio of 20:40:40% v/v in isocratic mode. Chromatogram of standard solution containing Adapalene is shown in Figure No.2. The developed HPLC method for the estimation Adapalene was validated as per the ICH guideline in terms of specificity, linearity, accuracy, precision, ruggedness and robustness, limit of detection and limit of quantification.

**Specificity**

The specificity of the method was determined by spiking the solution of placebo with the working standard solution containing Adapalene and this solution was analyzed as per the method described. The recorded chromatogram was compared with chromatogram of standard solution containing Adapalene to check the interference of the placebo with the response produced by the Adapalene.

**System suitability**

The system suitability of the method was determined by five replicate analysis of the standard solution containing Adapalene and this solution was analyzed as per the method described. The reproducibility of peak area, retention time, theoretical plate and tailing factor of the peak of Adapalene were checked.

**Linearity**

The linearity of the method was assessed by analyzing the standard solution containing Adapalene at 5 different levels 50%, 80%, 100%, 120% and 150% of its working concentration. The calibration curve of peak area (vs) concentration was plotted and correlation coefficient and regression line equation for both drugs were determined. The calibration curve of Adapalene is shown in Figure No.3.
Accuracy
Accuracy of the method was assessed by analyzing the solutions containing Adapalene at three different levels of its working concentrations 50%, 100% and 150% of its working concentration. Standard solutions were spiked with placebo and the percentage recovery of the drugs from the placebo was calculated.

Precision
Intra-day precision was determined by carrying out three independent assays of Adapalene at three different time points on the same day. Inter-day precision of the method was determined by analyzing of Adapalene at three different time points on different days. The % RSD of the obtained results was calculated.

Ruggedness and robustness
The ruggedness of the method was ascertained by carrying out the assay of the sample on different instrument by different analyst using different column of similar types. Robustness of the method was determined by analyzing the sample by deliberately changed chromatographic conditions such as change in mobile phase composition (±2 ml), flow rate (0.1 ml/min) and detection wavelength (±2 nm).

LOD and LOQ
The limit of detection and limit of quantification of Adapalene were calculated by using standard deviation of the responses and the slope of the calibration curve of Adapalene. LOD and LOQ were estimated by using the following formula,

\[ \text{LOD} = \frac{(3.3 \times \sigma)}{S} \]
\[ \text{LOQ} = \frac{(10 \times \sigma)}{S} \]

Where \( \sigma \) is the standard deviation of the response
\( S \) is the slope of the calibration curve

Analysis of Adapalene in Tablet formulation
For the assay of Adapalene in Pharmaceutical formulations, a quantity of sample equivalent to 20 mg of Adapalene was transferred in to 100 ml volumetric flask and 20 ml of Tetra Hydrofuran was added. The content of the flask was sonicated for 10 minutes and the volume was then made up to 100 ml with Tetra Hydrofuran. This solution was filtered through the Whatman filter paper and 5ml of the filtrate was diluted to 50ml with diluent. Form the resulting solution 25 µl was injected in to the column and response was recorded under the same chromatographic conditions. Six such samples were prepared and analyzed in the same manner. The amount of Adapalene present in the sample was determined by comparing the mean peak area of sample with that of standard.

RESULT AND DISCUSSION
A simple, accurate and precise RP HPLC method was developed for the estimation of Adapalene in Pharmaceutical formulations. Specificity of the method was tested by comparing the response of blank, standard and placebo mixed sample solution. No interference of placebo was detected at the retention time of Adapalene. The system suitability tests were carried out to evaluate the resolution and reproducibility of the system for the analysis. The results of the system suitability test were summarized in Table No.1. Linearity of the method was evaluated at 5 different concentration levels 10- 30 µg/ml of standard solution of Adapalene. Adapalene was found to give linear detector response in the concentration under study with correlation coefficient of 0.9997. Accuracy of the method was determined by recovery test. The percentage recovery was found to be in the range of 98.20% - 101.70% of Adapalene in Table No.2. All results indicate that the method is highly accurate. This method was validated for its inter-day and intra-day precision. The results obtained were within the acceptable limit. The ruggedness and robustness of the method were determined and the % RSD of the results were found to be less than 2.0%, which demonstrate that the developed method is rugged and robust. Detection limit for Adapalene was 0.21 µg/ml quantification limit was 0.63 µg/ml which suggest that a nanogram level of the drug can be estimated accurately. All the results of validation parameters are summarized in the Table No.3.
### Table No.1: Results of System Suitability Parameters for the analysis of Adapalene

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters*</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Peak area</td>
<td>435897</td>
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<tr>
<td>2.</td>
<td>Theoretical Plates</td>
<td>2524.1</td>
</tr>
<tr>
<td>3.</td>
<td>Tailing Factor</td>
<td>0.95</td>
</tr>
<tr>
<td>4.</td>
<td>Retention time (minutes)</td>
<td>10.42</td>
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<tr>
<td>5.</td>
<td>% RSD of Peak area</td>
<td>0.351</td>
</tr>
</tbody>
</table>

*Mean of six determinations

### Table No.2: Results of Recovery Studies of Adapalene

<table>
<thead>
<tr>
<th>S. No</th>
<th>Level of Recovery</th>
<th>Amount of drug added in µg/ml</th>
<th>% Recovery*</th>
<th>± RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50%</td>
<td>10.2</td>
<td>100.61</td>
<td>0.236</td>
</tr>
<tr>
<td>2</td>
<td>100%</td>
<td>20.0</td>
<td>98.67</td>
<td>0.341</td>
</tr>
<tr>
<td>3</td>
<td>150%</td>
<td>30.3</td>
<td>100.25</td>
<td>0.524</td>
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</tbody>
</table>

*Mean of six determinations

### Table No.3: Results of Validation of the developed HPLC Method

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters*</th>
<th>Results</th>
</tr>
</thead>
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<td>1</td>
<td>Linearity (µg/ml)</td>
<td>10-30</td>
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<tr>
<td>2</td>
<td>Correl. coefficient</td>
<td>0.9994</td>
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<tr>
<td>3</td>
<td>% Recovery</td>
<td>98.20% to 101.70%</td>
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<tr>
<td>4</td>
<td>Inter-day precision (% RSD)</td>
<td>0.654</td>
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<tr>
<td>5</td>
<td>Intra-day precision (% RSD)</td>
<td>1.23</td>
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<tr>
<td>6</td>
<td>Robustness (%RSD)</td>
<td>0.742</td>
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<td>7</td>
<td>Ruggedness (%RSD)</td>
<td>0.369</td>
</tr>
<tr>
<td>8</td>
<td>LOD(µg/ml)</td>
<td>0.21</td>
</tr>
<tr>
<td>9</td>
<td>LOQ(µg/ml)</td>
<td>0.63</td>
</tr>
</tbody>
</table>

*Mean of six determinations
Figure No.1: Chemical structure of Adapalene

Figure No.2: Typical HPLC Chromatogram of Adapalene
CONCLUSION
The developed RP HPLC method for the estimation of Adapalene offers simplicity, selectivity, precision and accuracy. So the developed method can be used for the routine analysis of Adapalene in bulk and Pharmaceutical formulations.

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