A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Tretinoin in tablet dosage form. An Inertsil ODS C-18, 5µm column having 250 x 4.6mm internal diameter in isocratic mode with mobile phase containing acetonitrile: methanol: 0.1% ortho phosphoric acid in the ratio of 75:05:20 (v/v/v) was used. The flow rate was 1.0ml/min and effluents were monitored at 236nm. The retention time for Tretinoin was 7.005 min. The method was validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness. Limit of detection and limit of quantification were found to be 0.025ppm and 0.0824ppm respectively and recovery of Tretinoin from tablet formulation was found to be 99.27%. The proposed method was successfully applied for the quantitative determination of Tretinoin in tablet formulation.

**Key Words:** Tretinoin, HPLC, Linearity, Validation, Robustness.
INTRODUCTION

Tretinoin is the acid form of vitamin A and is also known as all-trans retinoic acid or ATRA. It is a drug commonly used to treat acne vulgaris and keratosis pilaris. It is available as a cream or gel. It is also used to treat acute promyelocytic leukemia (APL), and is sold for this indication by Roche under the brand name Vesanoid. IUPAC name is retinoic acid.

Molecular formula is C_{20}H_{28}O_{2}, Molecular mass is 300.4

Figure 1: Molecular Structure of Tretinoin

Literature survey revealed that numerous methods have been reported for estimation of Tretinoin in pharmaceutical formulations.

Present study involves development of LC method using simple mobile phase which is sensitive and rapid for quantification of Tretinoin in tablet dosage forms as well as subsequent validation of developed method according to ICH guide lines.

EXPERIMENTAL

Instrument

The liquid chromatographic system consisted of Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Intersil ODS C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing purpose.

REAGENTS AND MATERIALS

Methanol, Acetonitrile and Ortho phosphoric acid of HPLC grade was purchased from E.Merck, Mumbai, India.
PREPARATION OF STANDARD STOCK SOLUTION

A stock solution of Tretinoin was prepared by accurately weighing 10mg of drug, transferring to 100ml of volumetric flask, dissolving in 25ml of solvent and diluting up to mark with solvent. Appropriate aliquot of this solution was further diluted with solvent to obtain final standard solution of 10ppm of Tretinoin. Resultant solution was filtered through Ultipor N66 Nylon 6,6 membrane sample filter paper.

Preparation of sample Solution

The formulation tablets of Tretinoin were crushed to give finely powdered material. Powder equivalent to 10mg of drug was taken in 10 ml of volumetric flask containing 5ml of mobile phase and was shaken to dissolve the drug and then filtered through Ultipor N66 Nylon 6,6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent to obtain concentration of 10ppm.

![HPLC chromatogram of Tretinoin formulation](image)

**Figure 2: HPLC chromatogram of Tretinoin formulation**

CHROMATOGRAPHIC CONDITIONS

**Chromatographic conditions** The mobile phase consisting of acetonitrile : methanol : 0.1% ortho phosphoric acid were filtered through 0.45μm Ultipor N66 Nylon 6,6 membrane solvent filter, degassed and were pumped from the solvent reservoir in the ratio of 75:05:20(v/v/v), and was pumped into the column. The flow rate of mobile phase was maintained at 1.0ml/min and detection wavelength was set at 236nm with
a run time of 12min. The volume of injection loop was 20µl prior to injection of the drug solution the column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept in ambient temperature

**CALIBRATION CURVE**

Appropriate aliquots of standard Tretinoin stock solution were taken in different volumetric flasks and resultant solution was diluted up to the mark with mobile phase to obtain final concentration of 2, 4, 6, 8, 10ppm of Tretinoin. These solutions were injected into chromatographic system, chromatograms were obtained and peak area ratio was determined for each concentration of drug solution. Calibration curve of Tretinoin was constructed by plotting peak area ratio versus applied concentration of Tretinoin and regression equation was computed. Similarly the sample solution was chromatographed and concentration of Tretinoin in tablet sample was found out using regression equation.

**METHOD VALIDATION**

The method was validated for accuracy, precision, linearity, specificity, limit of detection, limit of quantification and robustness by following procedures.

**Accuracy**

The accuracy of the method was determined by calculating recovery of Tretinoin by the method of standard addition. Known amount of Tretinoin (4ppm, 4ppm and 8ppm) was added to a pre quantified sample solution and the amount of Tretinoin was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and the amount of Tretinoin was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated.

**Precision**

The intra-day precision study of Tretinoin was carried out by estimating the correspondence responses six times on the same day with 10ppm concentration and inter-day precision study of Tretinoin was carried out by estimating the correspondence responses six times next day with 10ppm concentration.

**Linearity and range**

The linearity of the method was determined at seven concentration levels ranging from 2-10ppm for Tretinoin.

**Specificity**

Commonly used excipients (colloidal silicon dioxide, lactose, magnesium stearate, povidone, starch and talc) were spiked into a pre-weighed quantity of drug. The chromatogram was taken by appropriate dilutions and the quantity of drug was determined.
Limit of detection and limit of quantification

Limit of detection = 0.025ppm

Limit of quantification = 0.0824ppm

Stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both the solutions were analyzed over a period of 8 hours at room temperature.

Robustness

Robustness of the method was studied by changing the composition of organic phase by ±5% and the pH by ± 0.2, and also by observing the stability of the drugs for 24 hours at ambient temperature in the mobile phase.

RESULTS AND DISCUSSION

The UV spectra of Tretinoin showed that the drug absorbs appreciably at 236nm was selected as the detection wave length in liquid chromatography. Optimization of mobile phase was performed based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Acetonitrile: Methanol: 0.1%Ortho phosphoric acid in the ratio of 75:05:20 (v/v/v) was used. The retention time of Tretinoin was found to be 7.005min, which indicates a good base line.

Figure 3: HPLC chromatogram of Tretinoin
The number of theoretical plates was found to be 5516, which indicates efficient performance of the column. The asymmetric factor was found to be 1.84, which indicates asymmetric nature of the peak. The calibration curve for Tretinoin was obtained by plotting the peak area ratio versus the concentration of Tretinoin over the range of 2-10ppm, and it was found to be linear with $r^2 = 0.999$. The regression equation of Tretinoin concentration over its peak area ratio was found to be $y = 4972.23 + 47223.82x$, where $x$ is the concentration of Tretinoin (ppm) and $Y$ is the respective peak area. The data of regression analysis of the calibration curve was shown in table 1. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The limit of detection and limit of quantitation for Tretinoin was found to be 0.025ppm and 0.0824ppm, indicates the sensitivity of the method. The system suitability and validation parameters were given in table 2. The high percentage of recovery of Tretinoin was found to be 99.27% indicates that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of Tretinoin in tablet formulation. The result for Tretinoin was comparable with a corresponding labeled amount (Table 3). The absence of additional peaks indicates no interference of the excipients used in the tablets.

Table 1: Regression analysis of the calibration curve

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration range (ppm)</td>
<td>2-10</td>
</tr>
<tr>
<td>Slope</td>
<td>47223.82</td>
</tr>
<tr>
<td>Intercept</td>
<td>4972.23</td>
</tr>
<tr>
<td>Correlation coefficient ($r^2$)</td>
<td>0.999</td>
</tr>
</tbody>
</table>

Table 2: System suitability and validation parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical plates (N)</td>
<td>5516</td>
</tr>
<tr>
<td>Retention time (min)</td>
<td>7.005</td>
</tr>
<tr>
<td>Asymmetric factor</td>
<td>1.84</td>
</tr>
<tr>
<td>LOD (ppm)</td>
<td>0.025</td>
</tr>
<tr>
<td>LOQ (ppm)</td>
<td>0.0824</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>99.31%</td>
</tr>
<tr>
<td>R.S.D. (%)</td>
<td>0.696%</td>
</tr>
</tbody>
</table>

Table 3: Assay results of formulation

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Labelled claim (gm)</th>
<th>% of Tretinoin in Tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinoin (ointment)</td>
<td>1gm</td>
<td>4.75%</td>
</tr>
</tbody>
</table>
CONCLUSION

Proposed study describes new LC method for the estimation of Tretinoin in tablet formulation and serum. The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore the proposed method can be used for routine analysis of estimation of Tretinoin in its tablet formulation and serum.

REFERENCES

1) MedlinePlus Drug Information: Tretinoin Topical