ABSTRACT
A Rapid and Precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the estimation of Nicergoline, in its pure form as well as in its tablet dosage form. Chromatography was carried out on a Phenomenex Gemini C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol and Phosphate buffer (pH 6, adjusted with potassium dihydrogen phosphate) in the ratio of 70:30 v/v as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 289 nm. The retention time of the Nicergoline was 2.212 min respectively. The method produced linear responses in the concentration range of 10-50µg/ml of Nicergoline. The method precision for the determination of assay was below 2.0% RSD. The method is useful in the quality control of bulk and pharmaceutical formulations. Stability indicating studies were also carried out as per standard procedures.

KEYWORDS: Nicergoline, RP-HPLC, stability indicating studies, validation, Tablet dosage form.

INTRODUCTION
Nicergoline is chemically [(8β) – 10 – methoxy - 1,6 – dimethylerygolin – 8 - yl]methyl 5-bromopyridine-3-carboxylate and it belongs to the dihydropyridine is an ergot derivative used to treat senile dementia and other disorders with vascular origins. It has been found to
increase mental agility and enhance clarity and perception. It decreases vascular resistance and increases arterial blood flow in the brain, improving the utilization of oxygen and glucose by brain cells. It has similar vasoactive properties in other areas of the body, particularly the lungs.

It is used for vascular disorders such as cerebral thrombosis and atherosclerosis, arterial blockages in the limbs, Raynaud's disease, vascular migraines, and retinopathy. The chemical formula for Nicergoline is C_{24}H_{26}BrN_{3}O_{3}. It is sparingly soluble in water. The chemical structure for Nicergoline is following on figure-1, and literature survey revealed that numerous methods have been reported for estimation of Nicergoline in pharmaceutical formulations has been reported. The main objective of the work was to develop simple, fast, inexpensive, sensitive and accurate methods which could be applied to analyse Nicergoline in pure form and in pharmaceutical dosage form.

![Chemical Structure of Nicergoline](image)

**Fig.01: Chemical Structure of Nicergoline**

**MATERIALS AND METHODS**

**Pharmaceutical formulation**

Tablets contained cholergol labeled 30mg of Nicergoline per tablet.

**Reagents and Materials**

Methanol HPLC grade, HPLC grade water was obtained by double distillation and purification through mille-Q water purification system.

**Preparation of Standard solution**

Standard stock solution of Nicergoline was prepared using methanol to get 1 mg/ml, further dilution was made by diluting 1 ml to 10 ml with mobile phase to obtain 100 μg/ml. For the construction of calibration graph, the aliquots of stock solution of Nicergoline (1-5 ml of 100 μg/ml) was diluted with mobile phase to get 10-50μg/ml. The solutions were injected and chromatograms were recorded. Beer's law obeyed in the concentration range of 10-50 μg/ml.
Preparation of Sample solution

For analysis of tablet formulation, the tablet powder equivalent to 100 mg of Nicergoline was taken in a clean dry volumetric flask and made up to 100 ml with methanol the solution was sonicated for 10 min and filtered through Whatmann filter paper No.41. From clear solution, further dilutions were made by diluting 10 ml to 100 ml with mobile phase to obtain 100 μg/ml. 3.0 ml of test solution (30 μg/ml) was taken in six 10 ml volumetric flasks and made up to mark with mobile phase. The test solutions (10 μg/ml) were injected and chromatograms were recorded. For recovery studies, to the preanalysed formulation, solutions of raw material containing different concentrations were added and the amount of drug recovered was calculated. To each 3.0 ml of preanalysed formulation solution (30 μg/ml) added 1, 2, 3ml of raw material stock solution 100 μg/ml into 10 ml volumetric flasks and made up with mobile phase. The procedure was repeated as per the analysis of formulation. The amount of drug recovered was calculated by using slope and intercept values from the calibration graph. Finally the method was validated as per ICH guide lines for precision, accuracy, specificity, linearity, reproducibility, LOD and LOQ.

Chromatographic Conditions

Prepare a mixture of 700ml(70%) of HPLC methanol and 300ml of Phosphate buffer(30%) sonicated to degasse and the solvents were pumped from the solvent reservoir in the ratio of Methanol, Phosphate Buffer pH6 (70:30 v/v) into the column. The flow rate of mobile phase was maintained at 1.0ml/min and detection wavelength was set at 289 nm with a run time of 20min. The volume of injection loop was 25μl prior to injection of the drug solution. The column was equilibrated for at least 25min with the mobile phase flowing through the system. The column and the HPLC system were kept in ambient temperature.

Table. 1: Optimized method parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile Phase</td>
<td>Methanol: Phosphate Buffer pH6 (70:30 v/v)</td>
</tr>
<tr>
<td>Column ( Stationary Phase )</td>
<td>Phenomenex Gemini C18 (4.6×250mm, 5µ)</td>
</tr>
<tr>
<td>Flow rate ( ml/min)</td>
<td>1</td>
</tr>
<tr>
<td>Volume of injection loop (µl)</td>
<td>10</td>
</tr>
<tr>
<td>Detection wavelength (nm )</td>
<td>289</td>
</tr>
<tr>
<td>Column temperature (°C)</td>
<td>Ambient</td>
</tr>
<tr>
<td>Run time</td>
<td>8 min</td>
</tr>
</tbody>
</table>
Calibration Curve

Appropriate aliquots of standard Nicergoline 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 10, 20, 30, 40, 50μg/ml of Nicergoline. 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 Nicergoline was constructed by plotting peak area ratio versus applied concentration of Nicergoline and regression equation was computed. Similarly the sample solution was chromatographed and concentration of Nicergoline in tablet sample was found out using regression equation. ($r^2 = y = 44845x + 60406$).

Method Validation

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

Accuracy

The accuracy of the method was determined by calculating recovery of Nicergoline by the method of standard addition. Known amount of Nicergoline was added to a pre quantified sample solution and the Amounts of Nicergoline are estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve (Table 1). The recovery studies were carried out three times over the specified concentration range and amount of Nicergoline 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.
Table. 2: The Accuracy results for NICERGOLINE

<table>
<thead>
<tr>
<th>% Concentration (at specification Level)</th>
<th>Area</th>
<th>Amount Added (ppm)</th>
<th>Amount Found (ppm)</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>735405</td>
<td>15</td>
<td>15.051</td>
<td>100.345</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>1414436</td>
<td>30</td>
<td>30.193</td>
<td>100.645</td>
<td></td>
</tr>
<tr>
<td>150%</td>
<td>2069957</td>
<td>45</td>
<td>44.811</td>
<td>99.580</td>
<td>100.19%</td>
</tr>
</tbody>
</table>

**Precision**

The intra-day precision study of Nicergoline was carried out by estimating the correspondence responses six times on the same day with 100µg/ml concentration and inter-day precision study of Nicergoline was carried out by estimating the correspondence responses six times next day with 100µg/ml concentration.

**Linearity and range**

The linearity of the method was determined at six concentration levels ranging from 10-50µg/ml for Nicergoline. Fig.3.

![Calibration curve for Nicergoline](image)

**Fig. 3: Calibration curve for Nicergoline**

Limit of detection-2.167µg/ml.
Limit of quantification-6.568 µg/ml

**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 flow rate of organic phase by ±1% and the PH by ±0.1, and also by observing the stability of the drugs for 24 hours at ambient temperature in the mobile phase.

RESULTS AND DISCUSSION

Selection of the detection wavelength

The UV spectra of Nicergoline in comprising mixture of buffer (pH 6, adjusted potassium dihydrogen phosphate) Methanol: Phosphate Buffer in the ratio of 70:30 v/v, was scanned in the region between 200 and 400 nm and shows λmax at 289 nm.

Optimization of the chromatographic conditions

Proper selection of the stationary phase depends upon the nature of the sample, molecular weight and solubility. The drug Nicergoline is polar. The mixture of methanol and phosphate buffer was selected as mobile phase and the effect of composition of mobile phase on the retention time of Nicergoline was thoroughly investigated. A short run time and the stability of peak asymmetry were observed in the ratio of Phenomenex Gemini C18 (4.6×250 mm, 5µ) column, with mobile phase comprising mixture of buffer (pH 6, adjusted potassium dihydrogen phosphate) Methanol: Phosphate Buffer in the ratio of 70:30 v/v, at the flow rate 1 ml/min. The detection was carried out at 289 nm. The retention time of Nicergoline was found to be 2.212 min, which indicates a good baseline (Figure-3). The number of Theoretical plates was found to be 6294. (USP) which indicates efficient performance of the column. The calibration curve for Nicergoline was obtained by plotting the peak area ratio versus the concentration of Nicergoline over the range of 10-50 μg/ml, and it was found to be linear with $r^2 = 0.999$. The regression equation of Nicergoline concentration over its peak area ratio was found to be $y = 100.00\%$, where $x$ is the concentration of Nicergoline and $Y$ is the respective peak area. The data of regression analysis of the calibration curve was shown in table. 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 quantification for Nicergoline was Found to be 2.167 μg/ml and 6.568 μg/ml, indicates the sensitivity of the method. The system suitability and validation parameters were given in (Table). The high percentage of recovery of Nicergoline was found to be 100.19% indicates that the proposed method is highly accurate. The absence of additional peaks indicates no interference of the recipients used in the tablets

FORCED DEGRADATION STUDIES
**Acid degradation**

20 tablets of Nicergoline were taken, powdered and the average weight of one tablet was calculated. From this powder of 100 mg equivalent weight of Nicergoline sample was taken into a 100mL clean dry volumetric flask and add about 30mL of 0.1N Hcl and kept a side for 3hours and add 30mL of 0.1N NaOH solution to neutralize the solution and volume was made up to mark by using Diluent and sonicated to dissolve it completely.

Further 0.15ml of Nicergoline above stock solution was pipetted out into a 10ml volumetric flask and diluted up to the mark with diluent. (Fig.4)

**Alkaline degradation**

20 tablets of Nicergoline were taken, powdered and the average weight of one tablet was calculated. From this powder of 100 mg equivalent weight of Nicergoline sample was taken into a 100mL clean dry volumetric flask and add about 30mL of 0.1N NaOH and kept a side for 3hours and add 30mL of 0.1N Hcl solution to neutralize the solution and volume was made up to the mark by using Diluent and sonicated to dissolve it completely.

Further 0.15ml of Nicergoline above stock solution was pipetted out into a 10ml volumetric flask and diluted up to the mark with diluent. Fig .5.

![Auto-Scaled Chromatogram](image_url)
Figure.5: showing Alkaline degradation for Nicergoline

**Thermal degradation**

20 tablets of Nicergoline were taken, powdered and the average weight of one tablet was calculated. From this powder of 100 mg equivalent weight of Nicergoline sample was taken into a 100mL clean dry volumetric flask and exposed to heat at 80-90°C for 3 hours and the volume was made up to the mark by using Diluent and sonicated to dissolve it completely.

Further 0.15 ml of Nicergoline above stock solution was pipetted out into a 10ml volumetric flask and diluted up to the mark with diluent. Fig .6.

Figure.6: showing Thermal degradation for Nicergoline

**Peroxide degradation**

20 tablets of Nicergoline were taken, powdered and the average weight of one tablet was calculated. From this powder of 100 mg equivalent weight of Nicergoline sample was taken into a 100mL clean dry volumetric flask and add about 30mL of Hydrogen peroxide solution and kept aside for 3 hours and volume was made up to the mark by using Diluent and sonicated to dissolve it completely.
Further 0.15ml of Nicergoline above stock solution was pipetted out into a 10ml volumetric flask and diluted up to the mark with diluent. (Fig 7)

![Auto-Scaled Chromatogram](image1)

**Figure.7: showing peroxide degradation for Nicergoline**

**Photolytic degradation**

20 tablets of Nicergoline were taken, powdered and the average weight of one tablet was calculated. From this powder of 100 mg equivalent weight of Nicergoline sample was taken into a 100mL clean dry volumetric flask and expose to sunlight for 3 hours and the volume was made up to mark by using Diluent and sonicated to dissolve it completely.

Further 0.15ml of Nicergoline above stock solution was pipetted out into a 10ml volumetric flask and diluted up to the mark with diluent. (Fig 8). The degradation values are shown in the (Table 3).

![Auto-Scaled Chromatogram](image2)

**Figure.8 showing Photolytic degradation for Nicergoline**
Table.3 Results for degradation studies of Nicergoline

<table>
<thead>
<tr>
<th>S. No</th>
<th>Type of degradation</th>
<th>Weight of sample (µg/ml)</th>
<th>Area of sample</th>
<th>Assay content(% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acid (0.1N HCl)</td>
<td>30</td>
<td>29.832</td>
<td>99.441</td>
</tr>
<tr>
<td>2</td>
<td>Base (0.1N NaOH)</td>
<td>30</td>
<td>29.422</td>
<td>98.073</td>
</tr>
<tr>
<td>3</td>
<td>Peroxide (3% H_2O_2)</td>
<td>30</td>
<td>29.190</td>
<td>97.300</td>
</tr>
<tr>
<td>4</td>
<td>Thermal (at 60°c)</td>
<td>30</td>
<td>28.796</td>
<td>95.988</td>
</tr>
<tr>
<td>5</td>
<td>Photolytic (sunlight)</td>
<td>30</td>
<td>29.632</td>
<td>98.775</td>
</tr>
</tbody>
</table>

Summary

RP-HPLC method was developed for estimation of Nicergoline in bulk and its tablets dosage form. Chromatographic separation was performed on Phenomenex Gemini C_{18} (4.6×250mm,5µ) column, with mobile phase comprising mixture of buffer (pH 6, adjusted potassium dihydrogen phosphate) Methanol: Phosphate Buffer in the ratio of 70:30 v/v, at the flow rate 1ml/min. The detection was carried out at 289 nm.

Table. 4 Summary for RP-HPLC Method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results obtained</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>System suitability</td>
<td>Nicergoline- 6294</td>
<td>Theoretical Plates –NLT 2000</td>
</tr>
<tr>
<td></td>
<td>Nicergoline- 0.83</td>
<td>Tailing Factor- NMT 2</td>
</tr>
<tr>
<td></td>
<td>Nicergoline- 2.212</td>
<td>Retention time</td>
</tr>
<tr>
<td>Precision</td>
<td>Nicergoline- 0.129 %</td>
<td>% RSD of Nicergoline- NLT 2</td>
</tr>
<tr>
<td>Linearity</td>
<td>Nicergoline-0.997</td>
<td>Correlation coefficient NLT 0.999</td>
</tr>
<tr>
<td>Accuracy</td>
<td>Nicergoline- 100.19 %</td>
<td>Percentage Recovery 98- 102%</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>Nicergoline-2.167µg/ml</td>
<td>-</td>
</tr>
<tr>
<td>Limit of quantitation</td>
<td>Nicergoline-6.568 µg/ml</td>
<td>-</td>
</tr>
</tbody>
</table>

CONCLUSION

The proposed RP-HPLC method was found to be precise, specific, accurate, rapid and economical for estimation of Nicergoline in tablet dosage form. The sample recoveries in all formulations were in good agreement with their respective label claims and this method can be used for routine analysis. It can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations and in routine laboratories.

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