DEVELOPMENT AND VALIDATION OF NICLOSAMID IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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Abstract: This assay method was development and validation of Niclosamide (NICLO) in bulk and pharmaceutical dosage form by RP-HPLC method. The drug separat by using RP-HPLC on a RP-Cosmosil C\textsubscript{18} (250 mm × 4.6 mm I.D.) with particle size 5 µm was selected. On the basis of literature survey the mobile phase was methanol +0.05% (TEA-OPA PH-3.0) Water, (40:60 % v/v), at flow rate 1 ml/min. The UV detector was operated the overlain spectra 254 nm was selected for the estimation of the drug. Linearity, accuracy, precision, Ruggedness and System suitability test was found to be acceptable concentration ranges 5 – 25μg/ml with a R\textsuperscript{2} 0.999 value respectively, in the drug.

KEY WORDS: Niclosamide, Validation, RP-HPLC, Simultaneous Estimation.

INTRODUCTION: Niclosamide works by killing tapeworms on contact. Adult worms (but not ova) are rapidly killed, presumably due to uncoupling of oxidative phosphorylation or stimulation of ATPase activity. The killed worms are then passed in the stool or sometimes destroyed in the intestine. Niclosamide may work as a molluscicide by binding to and damaging DNA. Niclosamide appears to be minimally absorbed from the gastrointestinal tract neither the drug nor its metabolites have been recovered from the blood or urine. Niclosamide is excreted in feces.
Material and method

Chemical and reagent
NICLO was kindly supplied as a gift sample by Swaroop Enterprises, Aurangabad. These drug was used as working standard. All the chemicals used were of HPLC grade (Merck Chem. Ltd., Mumbai) used without further purification. Double distilled water was used for mobile phase preparation.

Apparatus
The chromatographic system Agilent Technologies 1100 series (Gradient System) with a 20µL fixed loop and G1315D Diode array detector. The separation was performed on a RP- Cosmosil C\textsubscript{18} (250 mm ×4.6 mm I.D.) with particle size 5 µm was selected. Chromatographic data were recorded and processed using Chemstation.

Chromatography Conditions
Chromatographic separations of active substances were obtained by using RP- Cosmosil C\textsubscript{18} (250 mm ×4.6 mm I.D.) with particle size 5 µm. Mobile Phase is methanol +0.05% (TEA-OPA PH-3.0) Water, (40:60 % v/v), at flow rate of 1 mL/min. The detection at 254 nm. The total time of analysis was less than 10 min.

Standard solution
The stock standard solution of NICLO (20 μg/mL) was prepared by dissolving 10 mg of NICLO in 10 ml methanol (prepared solution is 1mg/ml) and take 0.2ml in 10ml with mobile phase Acetonitrile: Water (0.05% TEA (OPA pH3.0). Sample solution
Accurately weighed quantity of 10 mg (NICLO) were transferred to 10 ml volumetric flask containing 10 mL methanol and volume was adjusted up to mark. It was further diluted to get concentration 20 μg/ml of NICLO. Constant volume 20 μl was injected into column and peak area was recorded.

Selection of Detection wavelength
From the overlain spectra 254 nm was selected for the estimation of the drug simultaneously (Figure-1)

Validation of Proposed Method
Calibration curve (linearity)
From the stock standard solution, aliquots portions (10mg) were transferred into a series of 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentration in the range of 5-25μg/ml NICLO. A constant volume of 20 μl of each sample was injected with the help of Hamilton Syringe. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area versus the drug concentration.

Accuracy (% recovery)
It was done by recovery study using standard addition method at 80%, 100% and 120 % level; known amount of standard NICLO were added to pre-analyzed sample (20 μg/mL of NICLO) and subjected them to the proposed HPLC method.

Method precision (repeatability)
Precision of the method was verified by repeatability and intermediate precision studies.
Intra-day precision was studied by analyzing 10, 15, 20 μg/mL of NICLO for three times on the same day.
Inter-day precision was checked analyzing the same concentration for three different days over a period of week.

Repeatability was measured by analyzing 20 μg/mL of NICLO for five times.

**Robustness**

Robustness of the method was studied by making deliberate changes in few parameters *viz*; change in mobile phase composition, pH, and flow rate. The effects on the results were studied by injecting 20 μg/mL for NICLO; one factor was changed at one time to estimate the effect.

**Limit of detection (LOD) and limit of quantitation (LOQ)**

LOD and LOQ of the drug were calculated using the equations according to International Conference on Harmonization (ICH) guidelines.

**Specificity**

The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analytes in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analytes in presence of components that may be expected to be present in the sample matrix.

**System suitability test**

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing.

**Validation of the Proposed Method**

**Linearity**

The concentration in the range of 5-25 μg/ml NICLO separately. The linearity of calibration curves was found to be acceptable over the concentration ranges of for NICLO with a $R^2$ 0.999 values respectively.

**Accuracy**

The recoveries obtained were 102.04 % for NICLO, respectively (Table 2). The high values indicate that the method was accurate. The recovery studies showed that the results were within acceptable limits, above 99.5% and below 100.5%.

**Method precision**

Precision study was carried out using parameter like method repeatability study which showed that results were within acceptable limit 0.27 i.e. % RSD below 2.0 indicating that the method is reproducible. The results are shown in (Table No.2)

**LOD and LOQ**

LOD values for NICLO was found to be 0.416 μg/ml respectively. LOQ values for NICLO was found to be 1.261 μg/ml respectively.

**Robustness**

Robustness of the method was studied by making deliberate changes in few parameters *viz*; change in mobile phase composition, pH, and flow rate. standard deviation was found to be Bellow 1 and % RSD is less than 2 for all results.

**System Suitability Test**

A sample solution of 10 μg/ml of NICLO (n=5) was prepared and same was injected, then the system suitability parameters were calculated from the chromatogram. The parameters, retention times, resolution factor, tailing factor and theoretical plates were evaluated.
RESULTS AND DISCUSSION
The absorption spectra of NICLO significantly overlap. To optimize the LC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for NICLO was obtained with a mobile phase consisting of Methanol + 0.05% TEA (OPA pH 3.0) Water (40+60% v/v) 254 nm at flow rate 1.0 ml/min. Quantification of the drug was performed at 254 nm. Resolution of the components with clear baseline separation was obtained.

CONCLUSIONS
The proposed RP-HPLC method presented in this paper has compensations of simplicity, precision and convenience for separation and quantitation of NICLO in combination and can be used for the assay of their particular dosage form. Moreover, the proposed method is a stability indicating assay method that can determine NICLO.

ACKNOWLEDGEMENTS
The authors are thankful to Swaroop Enterprises, Aurangabad., for supplying API as generous gift sample.

Table 1. Regression analysis of the calibration curves for NICLO in the proposed HPLC Method.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NICLO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity Range (µg/mL)</td>
<td>5-25</td>
</tr>
<tr>
<td>Detection Wavelength (nm)</td>
<td>254</td>
</tr>
<tr>
<td>Slope ± SD</td>
<td>115.3</td>
</tr>
<tr>
<td>Intercept ± SD</td>
<td>2.493</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.999</td>
</tr>
</tbody>
</table>

SD- Standard deviation

Table 2. Summary of the validation parameters for the proposed HPLC method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NICLO</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD</td>
<td>0.416</td>
</tr>
<tr>
<td>LOQ</td>
<td>1.261</td>
</tr>
<tr>
<td>Accuracy</td>
<td>102.04</td>
</tr>
<tr>
<td>Repeatability (%RSD, n = 5)</td>
<td>0.19</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td></td>
</tr>
<tr>
<td>Inter-day, n = 5</td>
<td>102.59 (0.06)</td>
</tr>
<tr>
<td>Intra-day, n = 3</td>
<td>99.65 (0.64)</td>
</tr>
</tbody>
</table>

LOD = Limit of detection.
LOQ = Limit of quantification
RSD = Relative standard deviation.

Table 3. Assay results for the combined dosage form using the proposed HPLC method

<table>
<thead>
<tr>
<th>Formulation</th>
<th>NICLO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niclosan-500</td>
<td>99.58 ±0.27</td>
</tr>
</tbody>
</table>

SD = Standard deviation, 5 determinations
Table 4. System suitability test

<table>
<thead>
<tr>
<th>System suitability Parameters</th>
<th>NICLO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time ($t_R$)</td>
<td>5.688 min</td>
</tr>
<tr>
<td>Theoretical plate (N)</td>
<td>10882</td>
</tr>
<tr>
<td>Area</td>
<td>1537.73</td>
</tr>
<tr>
<td>Resolution</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 1: An $\lambda$ Spectra of NICLO.

![Figure 1: An $\lambda$ Spectra of NICLO.](image)

Figure 2: Chromatogram of NICLO.

![Figure 2: Chromatogram of NICLO.](image)
Figure 3. Calibration curve for NICLO

\[ y = 155.3x + 2.493 \]
\[ R^2 = 0.999 \]

References:

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