Method Development and Validation for the Estimation of Dothiepin Hydrochloride by using RP-HPLC in PURE and Tablet Dosage Form

Prasanthi Thayi1, Lakshman Rao Atmakuri2, Nandini Mandada3, Hemanth Mandava3, Bhuvaneswari Mandru3, Chaitanya Manne3

1Department of Pharmaceutical Analysis, Associate Professor, V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, Krishna District, Andhra Pradesh, INDIA.
2Department of Pharmaceutical Analysis, Professor and Principal, V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, Krishna District, Andhra Pradesh, INDIA.
3Student, V.V. Institute of Pharmaceutical Sciences, Gudlavalleru, Krishna District, Andhra Pradesh, INDIA.

ABSTRACT

Aim: A simple, sensitive, accurate and precise RP-HPLC method was developed for the determination of Dothiepin HCl (DTH) in pure and tablet dosage form. Methods: The method was developed by using Phenomenex C18 (250 X 4.6 mm, 5 µm) and the mobile phase composed of buffer (0.1M sodium acetate): acetonitrile in the ratio of 50:50 v/v. The buffer pH was adjusted to 2.8. The retention time for Dothiepin HCl was found to be 3.44 min. Linearity range for Dothiepin HCl was found to be 10-60 µg/mL and the regression equation was found to be y = 14691x - 12844. % RSD for intra- and inter-day precision was found to be 0.27% and 0.84%. Average mean recovery was found to be 99.94%. LOD and LOQ values obtained for Dothiepin HCl were found to be 0.825 µg/mL and 2.498 µg/mL respectively. Conclusion: The results are analysed statistically and are found to be satisfactory. Hence this method can be successfully employed for analysis of Dothiepin HCl in tablet dosage form. Key words: Dothiepin HCl, RP-HPLC, Linearity, Dosage form, Precision.

INTRODUCTION

Dothiepin HCl (Figure 1) formerly known as Dosulepin, is a tricyclic antidepressant drug prescribed for the treatment of depression of and associated anxiety/panic disorders. It is chemically (3E)-3-(6H-benzo[c] [1] benzothiepin-11-ylidene)-N, N-dimethylpropan-1-amine; hydrochloride.1-2 It is also useful in chronic pain disorders and insomnia. It acts as a Serotonin–Norepinephrine Reuptake Inhibitor (SNRI) and also has other activities including antihistamine, antiadrenergic, antiserotonergic, anticholinergic and sodium channel -blocking effects. Dothiepin HCl inhibits the reuptake of biogenic amines, increasing available neurotransmitter levels at the synaptic cleft. The use of Dothiepin is only recommended in patients who are intolerant or unresponsive to alternative antidepressant therapies.3-4 A survey of literature5-15 found that few HPLC methods were reported for estimation of Dothiepin HCl in pharmaceutical dosage forms. However the reported methods required long run time, hence there is an attempt has been made to develop a simple, rapid and accurate RP-HPLC method for estimation of Dothiepin HCl in tablet dosage form.

MATERIALS AND METHODS

Instrument

Agilent 1260 infinity binary pump HPLC with open lab software was used for chromatographic studies.
**Chemicals**

Dothiepin HCl was purchased from Spectrum Pharma Research Solutions, Hyderabad, India. HPLC grade acetonitrile, analytical grade sodium acetate, ortho phosphoric acid were purchased from E. Merck (India) Ltd., Mumbai. Dothiepin HCl tablets were purchased from local market. Triple distilled water was used throughout experiment.

**Dothiepin HCl standard stock preparation**

Weigh and transfer accurately about 100 mg of Dothiepin HCl working standard into a 100 mL clean dry volumetric flask, add about 20 mL of mobile phase, sonicate for 5 min and dilute to volume with mobile phase.

**Diluted standard**

Pipette out 1 mL of the Dothiepin HCl standard stock solution and dilute to 10 mL with mobile phase.

**RESULTS**

**Method development**

Initially method development was started with the selection of wavelength of detection. The UV spectrum of DTH in mobile phase was noted using UV spectrophotometer. The maximum absorbance was noticed at 230 nm. This wavelength was used for detection of DTH.

The proposed method was developed by several concurrent trails in order to establish the preferred chromatographic conditions which would be helpful to conduct a complete validation study. The mobile phase for consisting of 0.1M sodium acetate (pH 2.8): acetonitrile (50:50 v/v) at 1 mL/min flow rate and detection wave length 230 nm was optimized which gave sharp peak, minimum tailing factor with short run time for DTH. The retention time for DTH was found to be 3.44 min.

**Optimized chromatographic conditions**

**Buffer preparation**

Dissolve 1.3 g of sodium acetate in 100 mL of water. Adjusted the pH to 2.8 using ortho phosphoric acid and the solution is filtered and sonicated for 5 min.

Mobile phase: Acetonitrile: buffer (0.1M sodium acetate)

Ratio: 50:50 v/v

Column: ODS C\(_{18}\) (250×4.6 mm×5\(\mu\)m)

Wavelength: 230 nm

Flow rate: 1.0 mL/min

**Observation**

Peak was observed at 3.44 min and the peak shape was good with low asymmetric factor. Hence it was optimized.

The optimized chromatogram was shown in Figure 2.

**VALIDATION**

**System Suitability**

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated and the values are depicted in Table 1.

**Linearity**

Linearity was performed by preparing standard solutions of Dothiepin HCl at different concentration levels i.e., 5-15 \(\mu\)g/mL. The absorbance was measured at 230 nm. Each measurement was carried out in triplicate. Linearity was proven by regression analysis by the least square method. The straight line in the calibration curve (Figure 3) obeyed linearity in the concentration range of 5-15 \(\mu\)g/mL for Dothiepin HCl. The correlation coefficient, linearity results were presented in Table 2.

**Precision**

The precision of the method was confirmed by intra-day and inter-day analysis. The concentration used for the precision studies is 10 \(\mu\)g/mL and was assumed as 100%. To study the intra-day precision, the analysis of

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**Figure 1: Structure of Dothiepin HCl.**

**Figure 2: Optimized Chromatogram.**
The development and validation of a high-performance liquid chromatography (HPLC) method for the analysis of Dothiepin HCl is described. The method was developed for the determination of Dothiepin HCl in pharmaceutical dosage forms. The system suitability results for Dothiepin HCl are tabulated in Table 1, including retention time, peak area, theoretical plates, and tailing factor. Linearity results for Dothiepin HCl are provided in Table 2, showing the relationship between concentration and peak area. Table 3 presents the intra-day and inter-day results for Dothiepin HCl, indicating precision with mean peak areas, standard deviations, and %RSD. Table 4 details the accuracy results for Dothiepin HCl, demonstrating recovery at various spike levels. Ruggedness of the method was confirmed by analyzing samples prepared and injected by different analysts. Robustness was assessed by varying conditions such as flow rate and wavelength, comparing system suitability parameters with method precision.

Accuracy
Accuracy was performed in triplicate as per the test method with equivalent amount of Dothiepin HCl into each volumetric flask for each spike level to get the concentration equivalent to 50%, 100% and 150% of the labeled amount as per the test method. The average % recovery was calculated. The accuracy results were tabulated in Table 4.

Ruggedness
Ruggedness of the method was confirmed by the analysis of samples prepared and injected by different analysts. Samples of Dothiepin HCl at 10 µg/mL concentration were analyzed by different analysts. It was observed that there were no marked changes in absorbance, which demonstrated that the developed method was rugged in nature.

Robustness
To demonstrate the robustness of the method, prepared solution as per the test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision. The robustness results were furnished in Table 5.
Table 5: Robustness results of Dothiepin HCl.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameter</th>
<th>Optimised</th>
<th>Used</th>
<th>Rt (min)</th>
<th>Peak area</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flow rate</td>
<td>1 mL/min</td>
<td>0.8 mL/min</td>
<td>3.51</td>
<td>924936</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.0 mL/min</td>
<td>3.44</td>
<td>924684</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.2 mL/min</td>
<td>3.41</td>
<td>925382</td>
<td>0.95</td>
</tr>
<tr>
<td>2</td>
<td>Wavelength</td>
<td>230 nm</td>
<td>232 nm</td>
<td>3.42</td>
<td>914685</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>230 nm</td>
<td>3.44</td>
<td>925146</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>228 nm</td>
<td>3.46</td>
<td>923214</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Table 6: LOD and LOQ of Dothiepin HCl.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Measured value (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of detection</td>
<td>0.825</td>
</tr>
<tr>
<td>Limit of quantification</td>
<td>2.498</td>
</tr>
</tbody>
</table>

Table 7: Assay results of Dothiepin HCl formulation.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Label claim</th>
<th>Amount found</th>
<th>%Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOTHEP</td>
<td>50 mg</td>
<td>49.62 mg</td>
<td>99.24</td>
</tr>
</tbody>
</table>

Table 8: Method Validation Summary.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameter</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>System suitability</td>
<td>The %RSD for retention time is 0.19% and for peak area is 1.17%</td>
</tr>
<tr>
<td>2</td>
<td>Precision</td>
<td>%RSD for Intra and Inter-day Precision 0.27 and 0.84</td>
</tr>
<tr>
<td>3</td>
<td>Accuracy</td>
<td>%Mean recovery is between 99.66-100.32%</td>
</tr>
<tr>
<td>4</td>
<td>Linearity</td>
<td>Regression equation: $y=14691x-12844; R^2=0.999$</td>
</tr>
<tr>
<td>5</td>
<td>LOD and LOQ</td>
<td>LOD: 0.825 µg/mL and LOQ: 2.498 µg/mL</td>
</tr>
<tr>
<td>6</td>
<td>Ruggedness</td>
<td>%RSD value is below 2%</td>
</tr>
<tr>
<td>7</td>
<td>Robustness</td>
<td>The % variation change in wavelength and flow rate is within limits</td>
</tr>
</tbody>
</table>

Limit of detection and Limit of quantification (LOD and LOQ)

LOD and LOQ values are calculated from calibration curve method and the LOD and LOQ of Dothiepin HCl is given in Table 6.

Estimation of Dothiepin HCl tablet dosage forms

5 tablets of Dothiepin HCl (Dothe) were weighed and crushed them to a fine powder and a quantity of tablet powder equivalent to 50 mg of DTH was transferred to 50 mL volumetric flask and dissolved in mobile phase and the volume was adjusted up to the mark with mobile phase. The mixture was allowed to stand for 30 min with intermittent sonication to ensure complete dissolution. The resulting solution was filtered through a 0.22 µm membrane filter. The filtrate was diluted further with mobile phase to get the working sample solution. The assay results were shown in Table 7 and sample chromatogram was represented in Figure 4.

Discussion

The present RP-HPLC method was developed for estimation of Dothiepin HCl by using Phenomenex C₈ (250 x 4.6 mm, 5 µm) as stationary phase with mobile phase containing mixture of 0.1M sodium acetate (pH 2.8) and acetonitrile (50:50 v/v). The eluted compound was monitored at 230 nm. Dothiepin HCl peak was eluted at 3.44 min. The developed method was validated for parameters of specificity, linearity, precision, accuracy, limit of detection, limit of quantification and robustness as per approved ICH guidelines and validation results are summarized in Table 8.

Conclusion

A new, reversed-phase HPLC method has been developed for analysis of Dothiepin HCl in commercial formulation. The developed RP-HPLC method was found to be simple, accurate, sensitive and precise proving reliability of the method. The method is very simple and involving no complicated sample preparations. The run time was relatively short, i.e. 3.44 min, which enables rapid quantitation of many samples in routine and quality control analysis of formulations. The optimized solvent system was used throughout the experimental work and no interference from any excipient was observed. The developed RP-HPLC method was validated as per the ICH guidelines. These results have shown that method could find practical application as a quality-control tool for analysis of Dothiepin HCl in pharmaceutical dosage forms in quality-control laboratories.
ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS


REFERENCES

2. The Indian Pharmacopoeia. Indian Pharmacopoeia Commission, Ghaziabad, India. 2014;1619-20.

PICTORIAL ABSTRACT

• RP-HPLC method was developed and validated for the assay of Dothiepin HCl in tablet formulation. Analysis of Dothiepin HCl was carried out by using Phenomenex C18 (250 x 4.6 mm, 5 μm) as stationary phase with mobile phase containing mixture of 0.1M sodium acetate (pH 2.8) and acetonitrile (50:50 v/v) and the compound was detected in very short time i.e 3.44 min. The method was validated as per ICH guidelines and the results were satisfactory. So the developed method was simple, precise, accurate and appropriate for the determination of Dothiepin HCl in bulk and tablet dosage forms.

ABOUT AUTHORS

Ms. Prasanthi Thayi1: Associate Professor, Department of Pharmaceutical Analysis, V. V. Institute of Pharmaceutical Sciences, Gudlavalleru Krishna District.

M Nandini, has completed B. Pharmacy in V. V. Institute of Pharmaceutical Sciences, Gudlavalleru Krishna District.
Dr. A. Lakshmana Rao: he is currently the Principal of V. V. Institute of Pharmaceutical Sciences, Gudlavalleru. He has 18 years of Teaching, Research and administrative experience for B.Pharmacy, M.Pharmacy, Pharm.D. and Ph.D. Programs. He has 238 publications in various reputed national and international journals. His academic achievements include 8 guest lectures, authored 2 books, guided 8 candidates for Ph.D. and five are currently under his guidance for Ph.D. He has filed a patent on Preparation of Rosuvastatin Formulation for Treating Human Oral Squamous Cell Carcinoma. He is Ratified as Principal by Jawaharlal Nehru Technological University-Kakinada, Kakinada, as an Associate Professor by Andhra University, Visakhapatnam. He is Approved Research Director for Ph.D. Programmes, adjudicator of Ph.D. and M.Pharmacy Thesis in Pharmaceutical Sciences of various Universities in India and Abroad.

M Hemanth, completed B. Pharmacy in V. V. Institute of Pharmaceutical Sciences, Gudlavalleru Krishna District.

M Bhuvaneswari, has completed B. Pharmacy in V. V. Institute of Pharmaceutical Sciences, Gudlavalleru Krishna District.

M Chaitanya, has completed B. Pharmacy in V. V. Institute of Pharmaceutical Sciences, Gudlavalleru Krishna District.