Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Haloperidol and Trihexyphenidyl in API and Combined Tablet Dosage Form

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Trihexyphenidyl and Haloperidol, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Altima C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 225 nm. The retention time of the Trihexyphenidyl and Haloperidol was 2.102, 3.537±0.02min respectively. The method produce linear responses in the concentration range of 15-75ppm of Trihexyphenidyl and 37.5-187.5ppm of Haloperidol. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of pharmaceutical formulations.

Key words: trihexyphenidyl; haloperidol, RP-HPLC; validation; estimation

1. INTRODUCTION

Haloperidol is an antidyskinetic and antipsycotic drug whose IUPAC name is 1- cyclohexyl-1-phenyl-3-(1-piperidyl)-1-propanol. Molecular formula of Haloperidol is C21H23ClFNO2; the molecular weight is 375.864; the structural formula is shown below (GK Anthony et al., 2014).

Trihexyphenidyl also known as benzhexol and trihex, is an antiparkinsonian, anticholinergic and antihistaminic drug. It is used for the symptomatic treatment of Parkinson's disease and to treat extrapyramidal side effects occurring during antipsychotic treatment. It reduces the frequency and duration of oculogyric crises as well as of dyskinetic movements and spastic contractions (P. Subbareddy and TE Divakar, 2016). TrihexyphenidylHCl IUPAC name is 1-cyclohexyl-1-phenyl-3-(1-piperidyl) propan-1-ol hydrochloride (N Usha Rani et al., 2014) The molecular formula of TXH is C20H31NO.HCl; the
molecular weight is 337.93 g/mol; the structural formula is given below (Rudra Raju et al., 2014).

Several works are available for simultaneous estimation of Haloperidol and Trihexyphenidyl (Rudra Raju et al., 2014; AA Borkar et al., 2009). The aim of the present work is to develop and validate a simple RP-HPLC method for simultaneous estimation of Haloperidol and Trihexyphenidyl.

2. MATERIAL AND METHOD

The drug samples Haloperidol and Trihexyphenidyl were obtained from Sura Labs, Hyderabad. The solvents used were of HPLC grade methanol and water from Merck Co, Mumbai.

2.1 Instrumentation

The HPLC system used was WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA detector, Altima C18 (4.6×150mm, 5µ) column.

2.2 Chromatographic conditions

<table>
<thead>
<tr>
<th>Instrument used</th>
<th>Waters HPLC with auto sampler and PDA Detector 996 model.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>40°C</td>
</tr>
<tr>
<td>Column</td>
<td>Altima C18 (4.6×150mm, 5µ)</td>
</tr>
<tr>
<td>Buffer</td>
<td>Dissolve 1.5ml of Triethyl amine in 250 ml HPLC water and adjust the pH 4.5. Filter and sonicate the solution by vacuum filtration and ultra sonication.</td>
</tr>
<tr>
<td>pH</td>
<td>4.5</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Methanol: TEA buffer: ACN (50:25:25 v/v)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1ml/min</td>
</tr>
<tr>
<td>Wavelength</td>
<td>225 nm</td>
</tr>
<tr>
<td>Injection volume</td>
<td>10 µl</td>
</tr>
<tr>
<td>Run time</td>
<td>7 min</td>
</tr>
</tbody>
</table>

2.3 Preparation of Buffer and Mobile Phase

2.3.1 Preparation of Triethylamine (TEA) buffer (pH 4.5)

Dissolve 1.5ml of Triethyl amine in 250 ml HPLC water and adjust the pH 4.5. Filter and sonicate the solution by vacuum filtration and ultrasonication.

2.3.2 Preparation of mobile phase

Accurately measured 400 ml (40%) of Methanol, 200 ml of Triethylamine buffer (20%) and 400 ml of Acetonitrile (40%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

2.3.3 Diluent Preparation

The Mobile phase was used as the diluent.

2.3.4 Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Trihexyphenidyl and 10mg of Haloperidol working standard into a 10ml clean dry volumetric flask add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.45ml of the above Trihexyphenidyl and 1.125ml of the Haloperidol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

2.3.5 Preparation of Sample Solution

Take average weight of one Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Trihexyphenidyl and Haloperidol sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 1.125ml of the above sample stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

3. RESULTS AND DISCUSSION

Method was validated according to ICH Guidelines in terms of linearity, range, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ) (V. Tejasvi Reddy et al., 2016; K Ganesh et al., 2016).

3.1 System Suitability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits i.e., 1.09 for Trihexyphenidyl and 0.73 for Haloperidol.

3.2 Linearity

Linearity studies were done for five different concentrations of each drug. The five different concentrations covered 15-75 µg/ml for Trihexyphenidyl and 37.5-187.5 µg/ml for Haloperidol.
The calibration curve showed good linearity in the range of 15-75 µg/ml with correlation coefficient (r²) of 0.999 and regression equation of $y = 12066x + 13756$ for Trihexyphenidyl (Table 2). Calibration curve showed good linearity in the range of 37.5-187.5 µg/ml with correlation coefficient (r²) of 0.999 and regression equation of $y = 18768x + 33265$ for Haloperidol (Table 2).

**3.3 LOD and LOQ**

**3.3.1 Limit of detection**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$\text{LOD} = 3.3 \times \frac{\sigma}{S}$$

Where,

$\sigma = $ Standard deviation of the response  
$S = $ Slope of the calibration curve

**3.3.2 Limit of quantitation**

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$\text{LOQ} = 10 \times \frac{\sigma}{S}$$

Where,

$\sigma = $ Standard deviation of the response  
$S = $ Slope of the calibration curve

**Table 1: Summary of validation parameters for the proposed method**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Trihexyphenidyl</th>
<th>Haloperidol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity</td>
<td>15-75 µg/ml</td>
<td>37.5-187.5 µg/ml</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>13756</td>
<td>33265</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>12066</td>
<td>18768</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>LOD</td>
<td>0.8 µg/ml</td>
<td>6.9 µg/ml</td>
</tr>
<tr>
<td>LOQ</td>
<td>2.6 µg/ml</td>
<td>21.1 µg/ml</td>
</tr>
</tbody>
</table>

**3.4 Precision**

**3.4.1 Repeatability**

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

**3.4.2 Intermediate precision**

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions. The %RSD for the area of six replicate injections was found to be within the specified limits.

**Acceptance criteria**

%RSD of Six different sample solutions should not more than 2

**Table 2: Results of repeatability and intermediate precision of Trihexyphenidyl and Haloperidol**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Trihexyphenidyl</th>
<th>Haloperidol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Area</td>
<td>606665</td>
<td>4100.8</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>2542.3</td>
<td>0.18</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.42</td>
<td>0.18</td>
</tr>
<tr>
<td>Mean Area and SD</td>
<td>596209</td>
<td>2899.8</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>1718.7</td>
<td>0.13</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.29</td>
<td>0.13</td>
</tr>
</tbody>
</table>

**3.5 Accuracy**

To study accuracy of the proposed method, recovery studies were performed by spiking of standard drug solution to pre-analyzed sample at three different percentage concentrations 50, 100, 150%. The resultant solutions were then reanalyzed by the proposed method. At each percentage concentration six determinations were
performed. From the results obtained the method was found to be accurate. The percentage recovery and mean recovery were calculated and presented in table.

Table 3: Accuracy results for Trihexyphenidyl and Haloperidol

<table>
<thead>
<tr>
<th>Drug</th>
<th>%Concentration (at specification Level)</th>
<th>Area (ppm)</th>
<th>Amount Found (ppm)</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trihexyphenidyl</td>
<td>50%</td>
<td>287774</td>
<td>22.5</td>
<td>22.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>551495</td>
<td>45</td>
<td>44.8</td>
<td>98.6</td>
</tr>
<tr>
<td></td>
<td>150%</td>
<td>825175</td>
<td>67.5</td>
<td>67.42</td>
<td>99.5</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>1104782</td>
<td>56.25</td>
<td>56.249</td>
<td>100%</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>100%</td>
<td>2105321</td>
<td>112.5</td>
<td>112.48</td>
<td>99.9%</td>
</tr>
<tr>
<td></td>
<td>150%</td>
<td>3211306</td>
<td>168.75</td>
<td>168.75</td>
<td></td>
</tr>
</tbody>
</table>

3.6 Robustness

Robustness of the method was determined by changing flow conditions and variation of mobile phase organic conditions. From the results obtained the method was found to be robust.

Acceptance criteria

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

3.7 Formulation assay:

The proposed RP-HPLC method was validated by simultaneously determining Trihexyphenidyl and Haloperidol in pharmaceutical dosage form. Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

\[
%\text{ASSAY} = \frac{\text{Sample area} \times \text{Weight of standard} \times \text{Dilution of sample} \times \text{Purity} \times \text{Weight of tablet}}{\text{Standard area} \times \text{Dilution of standard} \times \text{Weight of sample} \times 100} \times 100
\]

The % purity of Trihexyphenidyl and Haloperidol in pharmaceutical dosage form was found to be 99.6% and 99.7% respectively.

4. CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Haloperidol and Trihexyphenidyl in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Haloperidol and Trihexyphenidyl was freely soluble in ethanol, methanol and sparingly soluble in water.

Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise.

5. REFERENCES


**Source of Support:** Nil  
**Conflict of Interest:** None declared