Development and Validation of UV Spectrophotometric Method for the Estimation of Haloperidol

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Authors’ contributions
This work was carried out in collaboration between both authors. Authors MY and UVSS designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author MY performed the experiments and statistical analysis. Both authors MY and UVSS managed the literature searches and analyses of the study. Both authors read and approved the final manuscript for publication.

ABSTRACT

**Aims:** The aim of the present work was to develop and validate a sensitive, simple, accurate, precise & cost effective UV spectrophotometric method for the estimation of haloperidol in prepared pharmaceutical formulations of solid lipid nanoparticles.

**Methodology:** The different analytical performance parameters such as linearity, range, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) were determined according to International Conference on Harmonization (ICH) Q2 (R1) guidelines. The study was performed in phosphate buffer of pH 7.4.

**Results:** The peak ($\lambda_{\text{max}}$) of haloperidol appeared at a wavelength of 247.5 nm in phosphate buffer (pH 7.4). Beer-Lambert’s law was obeyed in the concentration range of 2–20 μg/ml with correlation coefficient ($R^2$) 0.9994.

**Conclusion:** The results of the study demonstrated that the developed procedure was accurate, precise and reproducible, while being simple, cheap and less time consuming.

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Therefore, this method can be suitably applied for the estimation of haloperidol in prepared solid lipid nanoparticles.

**Keywords:** Antipsychotic; haloperidol; stability; UV spectrophotometric method; validation.

1. **INTRODUCTION**

Haloperidol is a dopamine inverse agonist of the typical antipsychotic class of medications that chemically belongs to butyrophenone group. It occurs as a white crystalline powder and chemically known as 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-4-piperidinol] with molecular weight of 375.86 g mol⁻¹. The chemical structure of haloperidol is shown in Fig. 1. Its mechanism of action is mediated by blockade of D2 dopamine receptors in brain. Being the antipsychotic drug, it is used to treat certain psychiatric conditions including schizophrenia, manic states, medicament induced psychosis and neurological disorders with hyperkinesias [1]. It is also used to treat extreme behavior problems in children and to ease the symptoms of Tourette’s syndrome. The dose of haloperidol for the treatment of schizophrenia is 5-15mg/day with an average of 10mg per day. Its therapeutic plasma concentrations are in the range of 4–20ng/ml. Most Common dosage forms are tablets and injections. Side effects related to haloperidol are extrapyramidal including acute dystonic reactions, akathisia syndrome, drug induced Parkinsonism, bradykinesia and tardive dyskinesia [2].

![Fig. 1. Structure of haloperidol](image)

Various analytical techniques have been used for determination of haloperidol in pharmaceutical formulations. These include high performance liquid chromatography (HPLC) [3], high performance thin-layer chromatography (HPTLC) [4], 19F NMR spectroscopy [5], square-wave adsorptive stripping voltammetry at a mercury electrode [6], square-wave and cyclic voltammetry at hanging mercury drop electrode [7], cyclic voltammetry at multi-walled carbon nanotubes-modified glassy carbon electrode [8]. Non aqueous titrimetric method also been developed for haloperidol determination [9]. UV spectrophotometric assay procedures have been developed and described in official compendia [10].

The analytical procedures based on UV spectrophotometry are still being frequently published and literature survey revealed that few spectrophotometric methods have been used to determine haloperidol in pharmaceutical preparations. These methods were based on the reaction with [Cr (NCS) 6]₃⁻, [BiI₆]³⁻ and picric acid, chloranilic acid, and p-chloranil. The peak (λmax) of haloperidol appeared at a wavelength of 245nm in methanol - 0.1M HCl mixture (9:1) and hence it was determined in commercial dosage forms by UV spectrophotometry [11]. Derivative spectrophotometric method has also been reported for quantitation of haloperidol in pharmaceutical preparations.
In this study, efforts were made to develop a simple & sensitive UV spectrophotometric method for the estimation of haloperidol in prepared pharmaceutical formulations of solid lipid nanoparticles (SLNs). The different analytical performance parameters such as linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) were determined according to ICH Q2 (R1) guidelines [12,13]. The develop UV spectrophotometric method was used for the determination of entrapment efficiency and drug loading capacity [14] [data not shown].

2. MATERIALS AND METHODS

2.1 Instruments

A Shimadzu UV–Visible spectrophotometer (UV -1800, Shimadzu Corporation, Kyoto, Japan) was used for all absorbance measurements with one cm matched quartz cells and Shimadzu electronic balance (AUX 220, Shimadzu Corporation, Kyoto, Japan) was used for weighing of all samples.

2.2 Materials

Haloperidol was received as a gift sample from Vamsi Labs Ltd. Solapur, Maharashtra (India), Potassium dihydrogen phosphate was purchased from Qualigens fine chemicals, Mumbai (India), and Sodium hydroxide was purchased from Fisher scientific, Mumbai (India). All chemicals and reagents used were of analytical grade. Double distilled water was used to prepare solutions wherever required and it was filtered before use through a 0.22 μm membrane filter.

2.3 Methods

2.3.1 Preparation of phosphate buffer pH 7.4

The phosphate buffer pH 7.4 was prepared as per the specifications given in Indian Pharmacopoeia [15].

2.3.1.1 Preparation of 0.2M potassium dihydrogen phosphate solution

27.218gm of potassium dihydrogen phosphate was dissolved in 1000ml of distilled water to produce 0.2M solution of potassium dihydrogen phosphate.

2.3.1.2 Preparation of 0.2M NaOH solution

8gm of sodium hydroxide was dissolved in 1000ml of distilled water to produce 0.2M sodium hydroxide solution.

2.3.1.3 Preparation of buffer

50ml of 0.2M potassium dihydrogen phosphate was placed in 200ml of volumetric flask, and then pH was adjusted to 7.40±0.05 by adding 39.1ml of 0.2M NaOH solution. Finally, the volume was made upto 200ml with distilled water and then filtered through 0.22μm membrane filter.
2.3.2 Determination of wavelength of maximum absorption

A standard stock solution of haloperidol (100μg/ml) was prepared by dissolving 10mg of drug in 10 ml of methanol in a 100ml of volumetric flask and then volume was made up to mark with phosphate buffer (pH 7.4). The dilutions of this stock solution (100μg/ml) were made by diluting the required aliquot with phosphate buffer to obtain standard solutions in the range of 2-20μg/ml. An UV spectroscopic scanning (200–400nm) was carried out with drug solutions to determine the wavelength of maximum absorption ($\lambda_{max}$) using same diluent as blank.

2.3.3 Validation procedure

Method was validated according to ICH Guidelines in terms of linearity, range, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ) [12].

2.3.3.1 Linearity and range

Linearity is the ability of the method to obtain test results that are directly proportional to analyte concentration within a given range. The range of an analytical method is the interval between the upper and lower concentration of analyte for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

To study the linearity, serial dilutions of haloperidol were suitably prepared in the concentration range of 2-20μg/ml in phosphate buffer (pH 7.4). The absorbance of each solution was scanned at 247.5nm using same diluent as blank. Calibration curve was constructed by plotting concentration versus absorbance on x and y axis respectively. Linearity was determined by regression equation. This experiment was repeated 3 times.

Range is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amount of analyte within or at the extremes of the specified range of the analytical procedure.

2.3.3.2 Precision

The precision was determined at two levels as per ICH, Q2 (R1) suggestions i.e. repeatability and intermediate precision.

Repeatability of drug sample was determined as intraday variation (3 concentrations/3 replicates each, three times a day/a minimum of 9 determinations covering the specified range for the procedure) whereas intermediate precision was determined by interday variation (for three different days) for the determination of haloperidol at three different concentration levels of 6, 12 and 18μg/ml in triplicate. The % relative standard deviation was calculated for absorbance to obtain the intraday variation and interday variation.

2.3.3.3 Accuracy as recovery studies

Accuracy is the closeness of the test results obtained by the analytical method to the true value. The method was further validated to check the sensitivity of the method to estimate haloperidol in the presence of excipients. The accuracy of the method was evaluated by standard addition method. The pre-analyzed samples of haloperidol (8μg/ml) were spiked with the extra 50%, 100% and 150%, of the standard drug and the mixtures were analyzed
by the proposed method. The experiment was performed in triplicate. The % recovery of each sample and % relative standard deviation was calculated at each concentration level.

2.3.3.4 Limit of detection and limit of quantitation

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can be detected, but not necessarily quantified as an exact value. The limit of quantitation (LOQ) is the lowest concentration of an analyte that can be quantitatively determined with acceptable precision and accuracy under the stated operational conditions of the method. LOD and LOQ of the drug were calculated using the following equations as per ICH guidelines.

\[
LOD = 3.3 \times \sigma/S \quad \ldots \ldots \ldots \ldots (1)
\]

\[
LOQ = 10 \times \sigma/S. \quad \ldots \ldots \ldots \ldots (2)
\]

Where \( \sigma \) = the standard deviation of the response; \( S \) = the slope of the regression line.

3. RESULTS AND DISCUSSION

3.1 Wavelength of Maximum Absorption

The wavelength of maximum absorption (\( \lambda_{\text{max}} \)) was found to be 247.5 nm in selected medium. It was also observed that there was no change in the \( \lambda_{\text{max}} \) of the drug in this concentration range (2-20 \( \mu \)g/ml) as shown in Fig. 2 by overlay spectra of drug.

Calibration curve was prepared in the concentration range of 2-20 \( \mu \)g/ml by plotting concentration and absorbance on X and Y axis respectively. Calibration curve data and calibration curve are shown in Table 1 and Fig. 3 respectively.

**Table 1. Calibration curve data for haloperidol in phosphate buffer (pH 7.4)**

<table>
<thead>
<tr>
<th>Concentration (( \mu )g/ml)</th>
<th>Mean absorbance at 247.5 nm ( \pm SD (n=3) )</th>
<th>Regressed absorbance</th>
<th>Equation of Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.103( \pm )0.006</td>
<td>0.1013</td>
<td>1. Equation of Line</td>
</tr>
<tr>
<td>4</td>
<td>0.178( \pm )0.010</td>
<td>0.1847</td>
<td>y=0.0417x+0.0179</td>
</tr>
<tr>
<td>6</td>
<td>0.261( \pm )0.011</td>
<td>0.2681</td>
<td>2. Correlation coefficient</td>
</tr>
<tr>
<td>8</td>
<td>0.362( \pm )0.002</td>
<td>0.3515</td>
<td>R(^2)=0.9994</td>
</tr>
<tr>
<td>10</td>
<td>0.440( \pm )0.006</td>
<td>0.4349</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.515( \pm )0.004</td>
<td>0.5183</td>
<td>3. Slope</td>
</tr>
<tr>
<td>14</td>
<td>0.607( \pm )0.004</td>
<td>0.6017</td>
<td>m=0.0417</td>
</tr>
<tr>
<td>16</td>
<td>0.691( \pm )0.002</td>
<td>0.6851</td>
<td>4. Intercept</td>
</tr>
<tr>
<td>18</td>
<td>0.768( \pm )0.002</td>
<td>0.7685</td>
<td>c=0.0179</td>
</tr>
<tr>
<td>20</td>
<td>0.846( \pm )0.003</td>
<td>0.8519</td>
<td></td>
</tr>
</tbody>
</table>
3.2 Method Validation

3.2.1 Linearity and range

The absorbance of the prepared dilutions (2-20μg/ml) was determined in triplicate and mean absorbance range (n=3) was found to be 0.103-0.846 with RSD values below 2% as shown in Table 1. The calibration curve obtained was evaluated by its correlation coefficient. The absorbance of the samples in the concentration range of 2.0-20µg/ml was linear with a correlation coefficient ($R^2$) 0.9994.
3.2.2 Precision

The precision was assessed by analyzing haloperidol in three different concentration levels as 6, 12 and 18 μg/ml of haloperidol in triplicate. The results of repeatability (intraday precision) and intermediate (interday) precision were expressed in the terms of % RSD. The intraday and interday precision study of the developed method confirmed adequate sample stability and method reliability where all RSDs were below 2% as shown in Table 2.

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>Mean absorbance at 247.5 nm± SD (n=3)</th>
<th>RSD (%)</th>
<th>Intermediate precision (interday)</th>
<th>Day</th>
<th>Mean absorbance at 247.5nm±SD (n=3)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.264±0.005</td>
<td>1.9</td>
<td>1</td>
<td>0.263±0.004</td>
<td>1.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>0.262±0.005</td>
<td>1.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>0.263±0.004</td>
<td>1.53</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.515±0.002</td>
<td>0.4</td>
<td>1</td>
<td>0.516±0.002</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>0.517±0.002</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>0.513±0.001</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>0.767±0.002</td>
<td>0.3</td>
<td>1</td>
<td>0.768±0.003</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>0.764±0.001</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>0.766±0.004</td>
<td>0.47</td>
<td></td>
</tr>
</tbody>
</table>

3.2.3 Accuracy as recovery studies

The standard addition technique was carried out by adding excipients (in the likely range to be used) to be used in the formulation development with the addition of drug at 4 (50%), 8 (100%) and 12 (150%) μg/ml concentrations in sample solution of 8 μg/ml.

The proposed method afforded recovery of 99.00-100.6% after spiking the additional standard drug solution to the previously analyzed test solution. The value of % recoveries and % RSDs are shown in Table 3. The high % recoveries indicated no interference of excipients that are used to prepare different formulations of haloperidol i.e. solid lipid nanoparticles.

<table>
<thead>
<tr>
<th>% of standard spiked to the sample</th>
<th>Concentration</th>
<th>% of drug recovered</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample (μg/ml)</td>
<td>Total including spiked sample (μg/ml)</td>
<td>Spiked sample Determined (μg/ml)±SD (n=3)</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>8</td>
<td>12</td>
<td>11.88±0.16</td>
</tr>
<tr>
<td>100</td>
<td>8</td>
<td>16</td>
<td>16.10±0.11</td>
</tr>
<tr>
<td>150</td>
<td>8</td>
<td>20</td>
<td>19.89±0.11</td>
</tr>
</tbody>
</table>
3.2.4 Limit Of Detection (LOD) and Limit Of Quantitation (LOQ)

LOD and LOQ of this method were determined by the standard deviation method. The value of LOD and LOQ were found to be 0.225 and 0.681 μg/ml respectively. The value of LOD and LOQ are shown in Table 4.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Absorption maxima (nm)</td>
<td>247.5</td>
</tr>
<tr>
<td>2.</td>
<td>Linearity range (μg/ml)</td>
<td>2-20</td>
</tr>
<tr>
<td>3.</td>
<td>Regression equation</td>
<td>y=0.0417x +0.0179</td>
</tr>
<tr>
<td>4.</td>
<td>Slope</td>
<td>0.0417</td>
</tr>
<tr>
<td>5.</td>
<td>Intercept</td>
<td>0.0179</td>
</tr>
<tr>
<td>6.</td>
<td>Correlation coefficient (R²)</td>
<td>0.9994</td>
</tr>
<tr>
<td>7.</td>
<td>Recovery (%)</td>
<td>99.00-100.6</td>
</tr>
<tr>
<td>8.</td>
<td>LOD (μg/ml)</td>
<td>0.225</td>
</tr>
<tr>
<td>9.</td>
<td>LOQ (μg/ml)</td>
<td>0.681</td>
</tr>
</tbody>
</table>

4. CONCLUSION

The results and the statistical parameters demonstrated that the proposed UV spectrophotometric method was simple, rapid, specific, accurate and precise. Therefore, this method can be used to determine haloperidol quantitatively for routine analysis in the prepared formulations of solid lipid nanoparticles without interference of commonly used excipients and related substances.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.
REFERENCES


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