RESEARCH ARTICLE

Determination of Pramipexole Dihydrochloride in Tablet Dosage Forms by Visible Spectrophotometric Method Using Acetyl Acetone-Formaldehyde Reagent
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Abstract
A simple and sensitive visible spectrophotometric method has been developed for the determination of pramipexole dihydrochloride (PPD). The method is based on the condensation of the drug with acetyl acetone and formaldehyde producing a yellow colored Hantzsch condensation product having maximum absorption at 455 nm. The method is optimized for pH, concentration of reagents (acetyl acetone and formaldehyde) required, temperature and heating time. Beer’s law was obeyed over the range of 5–150 μg mL⁻¹. Molar absorptivity, Sandell’s sensitivity, limit of detection and limit of quantification were calculated and were found to be $5.23 \times 10^3$ L mole⁻¹ cm⁻¹, 0.0849 μg cm⁻², 0.449 μg mL⁻¹, and 1.362 μg mL⁻¹, respectively. The method was applied to different tablet dosage forms containing PPD. The results of the proposed method were compared with the reported UV spectrophotometric method. The new method was found to be simple, accurate (t-test), and reproducible (F-test).

Keywords: Pramipexole dihydrochloride; Hantzsch reaction; spectrophotometric analysis; tablet dosage forms.

1. Introduction
Pramipexole dihydrochloride (PPD) [1–6], a nonergot dopamine agonist approved in the US (1997), is used as an antidyskinetic for the treatment of Parkinson’s disease. Its chemical name is (S)-N'-propyl-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine dihydrochloride (Figure 1). The ability of PPD to alleviate the signs and symptoms of Parkinson’s disease is supposed to be linked to its ability to stimulate dopamine receptors in the striatum.

![Figure 1: Structure of pramipexole dihydrochloride.](http://astonjournals.com/csj)
spectrophotometric methods are particularly attractive because of ease in accessibility and their quick applicability to routine analysis. Reagents used for spectrophotometric determination of PPD in bulk and pharmaceutical dosage forms include: N-(1-naphthyl) ethylenediamine hydrochloride (diazocoupling) [23], paradimethylaminobenzaldehyde (condensation reaction) [23], bromoresol green (ion pair complex formation) [24] and bromothymol blue (ion pair complex formation) [24]. The reported spectrophotometric methods suffer from one or more disadvantages like use of carcinogenic reagent, extraction with organic solvent, less sensitivity, and critical working conditions. Hence they are not employed for routine analysis.

The present paper, for the first time, describes a novel, sensitive, simple, accurate and precise visible spectrophotometric method for the determination of PPD in bulk and tablet dosage forms. The proposed method is based on the reaction of the PPD with acetylacetone-formaldehyde reagent to produce a yellow colored species having absorption maxima at 455 nm. The results of the analysis were validated by statistical analysis and recovery studies. Common additives used as excipients in the tablet dosage forms do not interfere in the determination of the PPD.

2. Methods

2.1. Instrumentation
All spectrophotometric measurements were carried out using an Elico (Hyderabad, India) double beam model SL 159 digital spectrophotometer. The cells used for absorbance measurements were 1-cm matched quartz cells. Elico (Hyderabad, India) LJ120 model pH meter was used for pH measurements. Kemi KWB 220 model water bath (Ernakulam, India) was used to control the temperature for color development. Samples were weighed by using Essae-Teraoka electronic weighing balance (Goa, India) PG1000 model.

2.2. Reagents
All chemicals used were of analytical reagent grade and used as received. Double distilled water was used in the preparation of all solutions. All the solutions were prepared afresh daily. Acetate buffer (pH 5) was prepared by dissolving 1.36 g of sodium acetate (Qualigens Fine Chemicals, Mumbai) and 6 mL of glacial acetic acid (Qualigens Fine Chemicals, Mumbai) in sufficient water to produce 100 mL. 8.4% Acetylacetone solution was freshly prepared by mixing 8.4 mL of acetyl acetone (Sdfine-Chem Limited, Mumbai) with 40 mL of acetate buffer (pH 5) and diluted to 100 mL with distilled water. 20% Formaldehyde was prepared by mixing 20 mL of 40% formaldehyde (Fisher Scientific, Mumbai) with 40 mL of acetate buffer (pH 5) and diluted to 100 mL with distilled water.

2.2.1. Standard solutions of pramipexole dihydrochloride
Pharmaceutical grade PPD was kindly gifted by Matrix Laboratories, Hyderabad, India, and was used as received. A stock standard solution containing 1 mg mL⁻¹ of PPD was prepared in water. Working standard solution equivalent to 500 (proposed method) and 400 (reference method) μg mL⁻¹ of PPD was obtained by appropriate dilution of stock solution with water.

2.3. Tablet dosage forms of pramipexole dihydrochloride
Tablet dosage forms of PPD such as Parpex (1 mg, Zydus Cadila, Ahmedabad) and Pramipex (0.5 mg and 1 mg, Sun Pharma, Mumbai) were purchased from the local pharmacy market.

2.4. Recommended procedure
Different aliquots (0.1–3.0 mL) of working standard (500 μg mL⁻¹) solutions containing 5–150 μg mL⁻¹ of PPD was transferred into a series of 10 mL volumetric flasks and the total volume was adjusted to 3 mL with acetate buffer (pH 5). To each flask, 2 mL of 8.4% v/v acetylacetone and 1 mL of 20% formaldehyde reagents were added. The flasks were stoppered and contents were mixed well. The mixture was heated at 70°C for 10 min, cooled and diluted to 10 mL with distilled water. The absorbance of the yellow color solution was measured at 455 nm against the reagent blank prepared similarly omitting the drug. The amount of PPD present in the sample was computed from the corresponding calibration curve.

2.5. Reference method

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Different aliquots of working standard solution (0.1–1.5 mL, 400 μg mL⁻¹) of PPD were transferred into a series of 10 mL calibrated flasks and diluted to the mark with water. The absorbance of the solution was measured at 260 nm against the water. The standard calibration curve was prepared to calculate the amount of PPD in unknown samples.

2.6. Analysis of pramipexole dihydrochloride in tablet dosage forms
Fifty tablets were weighed accurately and ground into a fine powder. An amount of powder equivalent to 25 mg of PPD was weighed into a 25 mL volumetric flask; 15 mL of the water was added and shaken thoroughly for about 10 min. The volume was diluted up to the mark with the same solvent, mixed well and filtered using a quantitative filter paper. The filtered solution was appropriately diluted with water. Convenient aliquots were subjected to analysis by the recommended procedure and reference method.

3. Results and Discussion
Hantzsch reaction [25] is a condensation reaction that allows the formation of dihydropyridine derivatives by condensation of an aldehyde with two equivalents of a β-ketoester in the presence of ammonia. In the same manner, acetylacetone (β-ketoester) together with formaldehyde (aldehyde) reacts with aliphatic amines by Hantzsch reaction forming a yellow colored product that can be measured spectrophotometrically or spectrofluorimetrically. The quantification of certain sulpha drugs [26] (sulfacetamide sodium, sulfadiazine, sulfadimidine, and sulfathiazole), kanamycin [27], lisinopril [28], gabapentin [29], cefrozil [29], tranexamic acid [30], and sitagliptin phosphate [31] in its bulk and pharmaceutical dosage forms were carried out using Hantzsch reaction.

The results obtained in the proposed method were based on the condensation of primary amino group of PPD with acetyl acetone, as β-diketone, and formaldehyde, as an aldehyde, producing a yellow colored product having maximum absorption at 455 nm (Figure 2). The probable reaction mechanism is shown in Figure 3.

3.1. Optimization of experimental variables
3.1.1. Effect of pH
The effect of pH on the Hantzsch condensation reaction is shown in Figure 4. This figure reveals that the absorbance of the Hantzsch condensation product increases in the media of pH from 3.5-5 and there is a decrease in the absorbance with the further rise in pH. Keeping in view the reproducibility and stability of the Hantzsch condensation product, a pH of 5 was considered optimum throughout the determination process.

![Figure 2: Absorption spectrum of Hantzsch condensation product.](http://astonjournals.com/csj)
3.1.2. Effect of acetylacetone

In order to fix the concentration of acetyl acetone, the absorbance of the solution containing fixed concentration of PPD (50 µg mL⁻¹) and 20% formaldehyde (1 mL) and varying volumes of 8.4% acetyl acetone (0.5–4.0 mL) was recorded in a media of pH 5. It is obvious from Figure 5 that the maximum absorbance was obtained with 2 mL of 8.4% acetyl acetone; above this volume, the absorbance remained unchanged. Therefore, 2 mL 8.4% acetyl acetone was considered was sufficient for the condensation reaction.
3.1.3. Effect of formaldehyde

In order to fix the concentration of formaldehyde, the absorbance of the solution containing fixed concentration of PPD (50 µg mL⁻¹) and 8.4% acetyl acetone (2 mL) and varying volumes (0.5–4.0 mL) of 20% formaldehyde was recorded in a media of pH 5. The results showed that 1 mL of 20% formaldehyde was sufficient for the condensation reaction (Figure 6). Thus, 1 mL of 20% formaldehyde was chosen for the procedure. The presence of excess of the formaldehyde does not alter the absorbance of the reaction.

3.1.4. Effect of temperature and heating time

No color was developed at room temperature. The effect of temperature was studied in the range of 30–100°C. The absorbance and reaction rate increased with increasing temperature up to 70°C, and higher temperature had negative effect (Figure 7). Therefore, 70°C was selected as the optimum temperature.

![Figure 5](http://astonjournals.com/csj)  
**Figure 5:** Effect of acetylacetone on the absorbance of Hantzsch condensation product.

![Figure 6](http://astonjournals.com/csj)  
**Figure 6:** Effect of formaldehyde on the absorbance of Hantzsch condensation product.

![Figure 7](http://astonjournals.com/csj)  
**Figure 7:** Effect of temperature on the absorbance of Hantzsch condensation product.
In order to obtain the highest and most stable absorbance, the effect of heating time on the condensation reaction was catalyzed by heating the reaction solution in a water bath at 70°C for 5–30 min. The time required for the completion of the reaction and maximum absorbance was obtained after 10 min of heating and remained constant up to 30 min (Figure 8). Therefore, the optimum heating time was fixed at 10 min.

3.2. Stability of Hantzsch condensation product
To study the stability of Hantzsch condensation product, the absorbance values of the reaction solution, after diluting, was measured at regular intervals of time. It was found that the absorbance remained constant at least for 4.5 hr. This suggests that the condensation product was stable for a period of 4.5 hr.

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3.3. Analytical data
Under the optimized experimental conditions, a series of solutions containing fixed concentration of the reagent and varying concentration of PPD in the media of pH 5 media was prepared. The absorbance values were measured at 455 nm against reagent blank solution. Linear analytical curve shown in the Figure 9 indicates that PPD can be determined in the concentration range 5–150 μg mL⁻¹. The analytical curve for the determination of PPD can be fitted into the equation: 

\[ A_{\text{455 nm}} = 0.0116C + 0.0309 \]

where \( A \) is absorbance and \( C \) is concentration of PPD in μg mL⁻¹.

The correlation coefficient was 0.9986. The molar absorptivity and Sandell’s sensitivity were found to be \( 5.23 \times 10^3 \) L mol⁻¹ cm⁻¹ and 0.0849 μg cm⁻² respectively. The limits of detection and quantification were found to be 0.449 and 1.362 μg mL⁻¹, respectively.

![Figure 9: Linearity curve for the proposed method.](image)

The intraday accuracy and precision of the proposed methods was determined by measuring the content of PPD in pure form at three different concentration levels (10, 80, and 140 μg mL⁻¹). The intraday precision and accuracy of the proposed method was performed by carrying out five independent analyses at each concentration level within one day. The results of standard deviation (SD), relative standard deviation (RSD), percent of error and recoveries by the proposed method in Table 1 reveal that the proposed method was reasonably accurate and precise.

<table>
<thead>
<tr>
<th>Concentration of PPD (μg mL⁻¹)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taken</td>
<td>Found ± SD (n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>9.95 ± 0.084</td>
<td>0.844</td>
<td>99.50</td>
</tr>
<tr>
<td>80</td>
<td>79.97 ± 0.592</td>
<td>0.740</td>
<td>99.96</td>
</tr>
<tr>
<td>140</td>
<td>140.10 ± 0.637</td>
<td>0.456</td>
<td>100.07</td>
</tr>
</tbody>
</table>

In order to further access the accuracy of the proposed method and to check the interference from excipients used in the formulations, recovery experiments were carried out by standard addition method. For this purpose, a known amount of pure PPD was added to pre-analyzed tablets and the total value of drug was estimated by the proposed method. The results (Table 2) were reproducible with low SD and RSD. No interference from the common pharmaceutical excipients was observed.

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Table 2: Experimental values obtained in recovery test for PPD tablets by proposed method.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Labeled claim (mg)</th>
<th>Concentration of PPD (mg)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Added</td>
<td>Found ± SD (n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parpex</td>
<td>1.0</td>
<td>0.50</td>
<td>1.47 ± 0.0057</td>
<td>0.387</td>
</tr>
<tr>
<td>Pramipex</td>
<td>1.0</td>
<td>0.50</td>
<td>1.52 ± 0.0064</td>
<td>0.421</td>
</tr>
<tr>
<td>Pramipex</td>
<td>0.5</td>
<td>0.25</td>
<td>0.78 ± 0.0035</td>
<td>0.448</td>
</tr>
</tbody>
</table>

3.4. Application of the proposed method

The proposed method was applied to the determination of PPD in two brands of tablets, Parpex (1 mg, Zydus Cadila, Ahmedabad) and Pramipex (0.5 mg and 1 mg, Sun Pharma, Mumbai). The results obtained by the proposed method were statistically compared with those of the literature (UV-Spectrophotometry) method [7] by applying Student’s t-test for accuracy and F-test for precision. The results of this study given in Table 3 reveal that the calculated t- and F-values did not exceed the tabulated values at the 95% confidence level, suggesting that the proposed method and the literature method did not differ significantly with respect to accuracy and precision.

Table 3: Determination of PPD in tablet dosage forms by proposed and reference methods.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Labeled claim (mg)</th>
<th>Found (mg) ± SD (n = 5)</th>
<th>t Value</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proposed method</td>
<td>Reference method</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parpex</td>
<td>1.0</td>
<td>1.020 ± 0.0086</td>
<td>0.995 ± 0.0036</td>
<td>1.38</td>
</tr>
<tr>
<td></td>
<td>% R = 102.0</td>
<td>% R = 99.50</td>
<td>RSD = 0.843</td>
<td>RSD = 0.361</td>
</tr>
<tr>
<td>Pramipex</td>
<td>1.0</td>
<td>0.998 ± 0.0049</td>
<td>0.997 ± 0.0028</td>
<td>1.43</td>
</tr>
<tr>
<td></td>
<td>% R = 99.80</td>
<td>% R = 99.70</td>
<td>RSD = 0.490</td>
<td>RSD = 0.280</td>
</tr>
<tr>
<td>Pramipex</td>
<td>0.5</td>
<td>0.495 ± 0.0073</td>
<td>0.502 ± 0.0038</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>% R = 99.50</td>
<td>% R = 100.40</td>
<td>RSD = 1.470</td>
<td>RSD = 0.756</td>
</tr>
</tbody>
</table>

R – Recovery.

*Tabulated t value at 95 % confidence level = 2.77 and Tabulated F value at 95% confidence level = 6.39.

4. Conclusion

A new visible spectrophotometric method based on the Hantzsch condensation of drug with acetylacetone-formaldehyde reagent was developed for the determination of PPD in tablet dosage form. The method is quite simple and do not require expensive or sophisticated instruments, pre-treatment of the drug, extraction with organic solvent and critical working conditions. The method has wider linear range with good precision and accuracy. Hence, the proposed method can be applied for routine analysis and in quality control laboratories for determination of PPD in bulk and tablet dosage forms.

Competing Interests

The authors declare that they have no competing interests.

Authors’ Contributions

PL carried out the analytical studies; APR carried out the statistical analysis; CBS drafted the manuscript; AR carried out the sequence alignment; KTS participated in analytical studies and helped to draft the manuscript.

References


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