CONdenSATION REACTIONS FOR THE ESTIMATION OF ENTACAPONE IN BULK DRUG AND ITS PHARMACEUTICAL FORMULATIONS

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ABSTRACT
Two simple, sensitive, selective, accurate, precise & economical methods (methods A and B) for the quantitative estimation of Entacapone in bulk drug & pharmaceutical formulation (Tablet) have been described in the present work. In method A and B the amino group of reduced Entacapone forms colored schiff’s bases with p-dimethylaminocinnmaldehyde (PDAC) & p-dimethylaminobenzaldehyde (PDAB) to yield orange red & yellow colored chromogen respectively, exhibiting absorption maximum at 524nm & 415 nm and obeying Beer’s law in the concentration ranges of 10-50 µg/ml, and 50-250 µg/ml respectively. The results of analysis for the two methods have been validated statistically & by recovery studies the results are compared with those obtained using UV spectrophotometric method

Key words: Spectrophotometric, Entacapone.

INTRODUCTION
Entacapone[1, 2] is a catechol-O-methyl-transferase (COMT) inhibitor used as an adjunct in the drug treatment of patients with Parkinson’s disease. Literature survey reveals that few methods like determination of Entacapone glycuronides in plasma serum or urine[3,4], It is also characterized in rat plasma by liquid chromatography Tandem mass spectrometry (LC-MS-MS)[5], Validation of U.V. spectrophotometric method for quantitative determination of Entacapone in tablets using experimental design of plackett Burman for Robustness evaluation and comparison with HPLC[6]. Synthesis of water-soluble prodrug of entacapone[7], Stability indication of LC determination of entacapone[8], RP HPLC in Pharmaceutical dosage forms[9]. There is no analytical report
for the estimation of Entacapone by using visible spectrophotometry in pharmaceutical formulations. In the present investigation two simple, accurate, economical, & sensitive visible spectrophotometric methods have been developed for the quantitative estimation of Entacapone in pure drug and its formulations using p-dimethylaminocinnmaldehyde (PDAC) in Method A and p-dimethylaminobenzaldehyde (PDAB) in Method B.

EXPERIMENTAL

Instrumentation

All spectral measurements were made on systronics 119 UV/Visible spectrophotometer.

CHEMICAL & REAGENTS

All the chemical used were of analytical grade.

1) P-dimethylaminocinnamaldehyde,(0.1% w/v in distilled ethanol)
2) P-dimethylaminobenzaldehyde (0.5 % w/v in distilled ethanol)
3) Hydrochloric acid(3N)
4) Zinc granules
5) distilled water.

Preparations of standard & sample solutions

Accurately weighed 100 mg of Entacapone (bulk drug or its formulation) was dissolved in 20 ml of alcohol and treated with 10 ml of 3 N Hcl & 500 mg of zinc granules. After keeping it for one hour with occasional shaking at room temperature, the solution was filtered through cotton wool, the residue was brought to 100 ml with distilled ethanol the concentration of reduced Entacapone was brought to 100 µg/ml by further dilution with ethanol.

Assay Procedure

Method A: Aliquots of Entacapone ranging from 1.0-5.0 ml (100µg /ml) were transferred into a series of 10 ml volumetric flasks. To each of these flasks was added 1 ml of PDAC reagent. This was mixed and kept aside for 2 minutes. The solution was made up to the volume with distilled ethanol and absorbance of orange red color chromogen was measured at 524nm against the reagent blank. The amount of Entacapone present in the sample was computed from its calibration curve. The colored species was found to be stable for more than 4 hours.
**Method B:** Aliquots of Entacapone ranging from 5.0-2.5 ml (100 µg /ml) were transferred into a series of 10 ml volumetric flasks. To each of these flasks were added 2 ml of PDAB reagent. This was mixed & kept aside for 2 minutes. The solution was made up to the volume with distilled ethanol & absorbance of yellow colored chromogen was measured at 415nm against the reagent blank. The amount of Entacapone present in the sample was computed from its calibration curve. The color species was found to be stable for more than 2 hours.

**TABLE 1: Optical Characteristics and Precision**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method-A</th>
<th>Method-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} ) (nm)</td>
<td>524</td>
<td>415</td>
</tr>
<tr>
<td>Beer’s law limits (µg/ml) (c)</td>
<td>10-50</td>
<td>50-250</td>
</tr>
<tr>
<td>Molar absorptivity (lit mol(^{-1}) cm(^{-1}))</td>
<td>5.437 x 10(^3)</td>
<td>8.181 x 10(^3)</td>
</tr>
<tr>
<td>Limit of detection (LOD) (µg ml(^{-1}))</td>
<td>2.748</td>
<td>4.28</td>
</tr>
<tr>
<td>Limit of quantification (LOQ) (µg ml(^{-1}))</td>
<td>8.32</td>
<td>12.93</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg/ml/0.001 abs units)</td>
<td>0.018</td>
<td>0.0263</td>
</tr>
<tr>
<td>Regression equation*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.0811</td>
<td>2.6829 x 10(^{-3})</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>1.0666 x 10(^{-3})</td>
<td>2.000 x 10(^{-4})</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9999</td>
<td>0.9999</td>
</tr>
<tr>
<td>Standard error of estimation (se)</td>
<td>3.4862 x 10(^{-3})</td>
<td>1.2302 x 10(^{-3})</td>
</tr>
<tr>
<td>Relative standard deviation**(%)</td>
<td>0.303</td>
<td>0.921</td>
</tr>
<tr>
<td>Confidence limits with 0.05 level</td>
<td>0.088</td>
<td>0.096</td>
</tr>
<tr>
<td>Confidence limits with 0.01 level</td>
<td>0.013</td>
<td>0.921</td>
</tr>
</tbody>
</table>

\*Y=bC+a, where C is the concentration of Entacapone in µg/mL and Y is the absorbance at the respective \( \lambda_{\text{max}} \).
\*Average of eight determinations
\**Average of three determinations
TABLE 2: Assay and Recovery of Entacapone in pharmaceutical Dosage Form

<table>
<thead>
<tr>
<th>Pharmaceutical dosage form</th>
<th>Labeled Amount</th>
<th>Amount obtained by the Proposed methods* (mg)</th>
<th>Reference method**(uv method)</th>
<th>Percentage recovers %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>200 mg</td>
<td>196.80±0.075</td>
<td>198.16±0.070</td>
<td>199.16±0.022</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t=0.011</td>
<td>t=0.0270</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F=0.086</td>
<td>F=0.095</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>200 mg</td>
<td>197.60±0.122</td>
<td>198.12±0.13</td>
<td>198.18±0.0027</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t=0.076</td>
<td>t=1.80</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F=0.048</td>
<td>F=0.231</td>
<td></td>
</tr>
</tbody>
</table>

*Average ± standard deviation of eight measurements, the t and F values refer to comparison of the proposed method with reference method. Theoretical value at 95% confidence limits t=2.365 and F=4.85
T1, T2 are tablets from different manufacturers Average of 5 determination (100 mg of Entacapone was added and recovered).

RESULT AND DISCUSSION

The optical characteristic such as Beer’s law, limits, Sandell’s sensitivity, molar extinction coefficient, percent relative standard deviation (Calculated from eight measurement containing 3/4th of the amount of the upper beer’s law limits) were calculated & the result are summarized in table1. Regression characteristic like slope, intercept, correlation-coefficient & percent range of errors (0.05 & 0.01 confidence limits) were also calculated & are shown in table 1.

Commercial formulation of Entacapone has been analyzed by the proposed method as well as UV methods and the results are presented in table 2. To evaluate validity and reproducibility of the methods, fixed amounts of the drug were added to the pre–analyzed formulations, these results of percentage recovery are summarized in table 2. There is no interference of additives & excipient in the proposed analytical methods. The proposed spectrophotometric methods for estimation of Entacapone are simple, sensitive, accurate & precise & can be used in routine estimation of this drug in bulk as well as in pharmaceutical formulation.
Reduction Zn/HCl

Reduction Entacapone

Orange red colored chromogen
\( \lambda \text{ max } 524 \text{ nm} \)
Method A

Yellow colored chromogen
\( \lambda \text{ max } 415 \text{ nm} \)
Method B
Drug Concentration 10 mcg/ml

![Graph of ENC - PDAC System and Reagent Blank Vs distilled ethanol]

**Figure 1**
Absorption Spectrum of ENC with PDAC and its reagent blank. $\lambda_{\text{max}}$ is at 524 nm

Drug Concentration 50 mcg/ml

![Graph of ENC - PDAB system and Reagent Blank Vs distilled ethanol]

**Figure 2**
Absorption Spectrum of ENC with PDAB and its reagent blank. $\lambda_{\text{max}}$ is at 415 nm

![Graph of Beer's law plot of ENC with PDAC system]

**Figure 3**
Beer’s law plot of ENC with PDAC system

![Graph of Beer's law plot of ENC with PDAB system]

**Figure 4**
Beer’s law plot of ENC with PDAB system
ACKNOWLEDGEMENT

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REFERENCE


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