DEVELOPMENT AND VALIDATION OF QUANTITATIVE SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF ENTACAPONE IN PHARMACEUTICAL FORMULATION

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ABSTRACT

In the present study, four sensitive, precise and accurate spectrophotometric methods have been developed for the determination of entacapone in pharmaceutical formulation. The first method (method I) is based on the reducing properties of entacapone to Folin-Ciocalteu’s phenol reagent (FCR) in alkaline medium producing blue color which exhibit $\lambda_{\text{max}} = 758$ nm. The second method (method II) is based on the presence of aketone group of entacapone to reacte with 2,4-Dinitrophenyl Hydrazine (2,4-DNPH) through hydrazine formation producing red color which exhibit $\lambda_{\text{max}} = 514$ nm. The third method (method III) is based on the reaction of entacapone with 4-Aminoantipyrine (4-AAP) in alkaline medium through formation of quinoid structure producing red color which exhibit $\lambda_{\text{max}} = 493$ nm. The fourth method (method IV) is based on the reduction of Red Tetrazolium by entacapone in alkaline medium into the corresponding highly colored formazan derivative producing red color with $\lambda_{\text{max}} = 486$ nm. The validation parameters like linearity, sensitivity, accuracy, precision and robustness were checked by following the ICH guidelines. The proposed methods were applied for the analysis of entacapone in tablet formulation with good results.

KEYWORDS: Entacapone, Folin-ciocalteu’s phenol reagent, 2,4-Dinitrophenyl Hydrazine, hydrazone, 4-Aminoantipyrine, quiniod structure, Red Tetrazolium and formazan derivative.
1-INTRODUCTION

Entacapone is chemically known as (2E)-2-cyano-3-(3,4-dihydroxy-5-nitrophenyl)-N,N-diethyl prop-2-enamide (Fig. 1). Entacapone is a selective and reversible catechol-O-methyl transferase (COMT) inhibitor for the treatment of Parkinson’s disease. It is a member of the class of nitrocatechols. When it is administered concomitantly with dopaminergic agents such as L-DOPA and a decarboxylase inhibitor (e.g., carbidopa), entacapone increases the bioavailability of these compounds by facilitating their passage across the blood-brain barrier.[1, 2] Entacapone is greenish yellow to yellow powder, sparingly soluble in acetone and in methanol, slightly soluble in ethanol, very slightly soluble tolune, practically insoluble in water.[3, 4, 5] Literature survey reveals that LC methods were reported for determination of entacapone in tablets and biological fluids.[6-11] Spectrophotometric methods were developed for the simultaneous determination of entacapone in bulk dosage form.[12-15]

The aim of the work is to introduce simple, accurate, selective, sensitive and unexpensive qualitative methods for determination of entacapone in pharmaceutical formulation.

![Chemical Structure of Entacapone](image)

C_{14}H_{15}N_{3}O_{5} M.wt. 305.286

Fig. (1): Structural formula of Entacapone

2. EXPERIMENTAL

2.1. Instruments

A double beam UV-Visible spectrophotometer Shimadzu 1650 (Tokyo, Japan), equipped with 10 mm matched quartz cells and it is connected to IBM compatible computer with the software UV-Probe Ver. 2.43. Water bath Arthermo (Italy) and pH- meter Jenway 3510 (England).
2.2. Materials and chemical reagents

All reagents were of analytical grades and solvents were of spectroscopic grade, water used was freshly distilled.

A. Entacapone was kindly provided by Sigma Pharmaceutical Company, Cairo, Egypt.
B. Parkicapone® tablet: (batch number 33019) manufactured by Al Andalous Medical Company; labeled to contain 200 mg of tentacapone per tablet.
C. Hydrochloric acid, Sodium carbonate (10 % aqueous soln.), Sodium hydroxide (1 N aqueous soln.), Potassium hydroxide (0.1 N in absolute ethanol), Ammonium chloride, Potassium ferricyanide (1% aqueous soln.) , Ammonium hydroxide (10 M aqueous soln.) and Methanol (El-Nasr Co., Egypt).
D. Folin-Ciocalteu’s phenol reagent (FCR) : (Sigma, USA) 2N solution.
E. 2,4-Dinitrophenyl Hydrazine (2,4-DNPH) : 0.1 % soln. in conc. HCl:methanol (1 : 10 v/v).
F. 4-Aminoantipyrine (4-AAP) : (Fluka Chemika Switzerland) 0.3% aqueous soln.
G. Red Tetrazolium : (Alfa Aesar, Germany) : 0.5% in absolute ethanol. This reagent is preferred to be freshly prepared.
H. Ammonia buffer (PH =10) (British pharmacopia 2006) ; prepared br dissolving 5.4 gm of ammonium chloride in 20 ml water , add 35 ml of 10 M ammonia soln. then dilute to 100 ml with water and adjust the required PH using PH meter.

2.3. Standard solutions: For method I : Stock solution of 100 µg /ml for entacapone was prepared by dissolving 10 mg of entacapone in 100 ml water : methanol (1:9 v/v).

For method II, III, IV : Stock solution of 100 µg /ml for entacapone was prepared by dissolving 10 mg of entacapone in 100 ml methanol. Different sets of working solution at various concentrations were prepared by appropriate dilution of the stock solution.

3. General procedures

3.1. Construction of calibration curves (linearity)

3.1.1. Method I: Aliquots of standard entacapone solution in water : methanol (1:9 v/v) (100 µg /ml) containing (10 – 80) µg of the drug were added to a series of 10 ml volumetric flasks followed by 1 ml 2 N FCR and 4 ml 10% sodium carbonate. The solutions were mixed and allowed to stand at room temperature for 35 min. Volumes were adjusted with water to the mark and the absorbance of the developed blue color was measured against reagent blank at 758 nm. (Fig. 2).
3.1.2. Method II

Aliquots of standard entacapone solution in methanol (100 µg/ml) containing (5 – 45) µg of the drug were added to a series of 20 ml screw capped test tubes followed by 2 ml 0.1% 2,4-DNPH. The tubes were mixed well and heated in water bath at 80-85 °C for 10 minutes. Then cooled, transferred quantitatively into a series of 10 ml volumetric flasks and 5 ml 1 N NaOH were added and then diluted to the mark with methanol. The absorbance of the developed color was measured against reagent blank at 514 nm. (Fig. 3).

Fig. (2): Absorption spectrum of entacapone (6 µg/ml) reaction product with FCR (- - -) its blank (....)

Fig. (3): Absorption spectrum of entacapone (3 µg/ml) reaction product with 2,4-DNPH (----) and its blank (....)
3.1.3. Method III
Aliquots of standard entacapone solution in methanol (100 µg /ml) containing (50 – 650) µg of the drug were transferred to a series of 25 ml volumetric flasks, 0.15 ml ammonia buffer was added followed by 1.5 ml 0.3% 4-AAP and 1.5 ml 1% potassium ferricyanide and the volumes were completed with water. The absorbance of the developed color was measured against reagent blank at 493 nm. (Fig. 4).

![Absorption spectrum of entacapone (18 µg/ml) reaction product with 4-AAP (----) and its blank (....)](image)

Fig. (4): Absorption spectrum of entacapone (18 µg/ml) reaction product with 4-AAP (----) and its blank (....)

3.1.4. Method IV
Aliquots of standard entacapone solution in methanol (100 µg /ml) containing (10 – 170) µg of the drug were transferred to a series of 10 ml volumetric flasks, followed by 3.5 ml 0.5% of Red Tetrazolium ethanolic soln. and 0.75 ml 0.1 N potassium hydroxide ethanolic slon., mixed well then the volumes were completed with ethanol. The soln. was allowed to stand for 40 minutes. at room temperature in a dark place. The absorbance of the developed color was measured against reagent blank at 486 nm. (Fig. 5).
3.2. Optimization of the Experimental Conditions

3.2.1. Method I

3.2.1.1. Effect of FCR volume

Into a series of 10 ml volumetric flasks, a definite concentration of entacapone equivalent to (60 µg) was allowed to react with different volumes of (2N) FCR soln. (0.25 – 2 ml) and 4 ml of 10% sodium carbonate was added, the reaction was allowed to proceed for 15 minutes at room temperature and volumes were adjusted with water. It was found that 1 ml of FCR was sufficient to give maximum absorbance (Fig. 6).
3.2.1.2. Effect of Na₂CO₃ volume

The procedure under (Effect of FCR volume) was repeated using 1 ml of FCR and different volumes of 10% Na₂CO₃ ranged from (1 – 6 ml). It was found that 4 ml of 10% Na₂CO₃ was sufficient to give maximum absorbance (Fig. 7).

![Graph showing the effect of Na₂CO₃ volume on absorbance.](image)

Fig. (7): Effect of volume of 10% sodium carbonate on the absorbance of entacapone (6 µg/ml) with FCR at 758 nm.

3.2.1.3. Effect of time

The procedure under (Effect of FCR volume) was repeated using 1 ml of FCR followed by 4 ml of 10% Na₂CO₃. Then the absorbance was allowed to be determined every 5 minutes. It was found that 35 minutes was sufficient to give maximum absorbance and the color was stable for more than one hour (Fig. 8).

![Graph showing the effect of time on absorbance.](image)

Fig. (8): Effect of time on the absorbance of entacapone (6 µg/ml) with FCR at 758 nm.
3.2.2. Method II

3.2.2.1. Effect of 0.1 % DNPH volume: A definite concentration of entacapone was allowed to react with different volumes of 0.1 % DNPH (0.5 – 2.5 ml) in a series of 20 ml screw capped test tubes. The tubes were heated in a water bath at 80 – 85 °C for 20 minutes and then cooled, transferred to 10 ml volumetric flasks, 4 ml of 1 N NaOH then the mixture was shaken and adjusted to volume with methanol. It was found that 2 ml of 0.1 % DNPH was sufficient to give maximum absorbance (Fig. 9).

![Fig. (9): Effect of 0.1% 2,4-DNPH volume on the absorbance of the reaction product with entacapone (3 µg/ml) at 514 nm.](image)

3.2.2.2. Effect of 1 N NaOH volume: The procedure under (Effect of 0.1 % DNPH volume) was repeated using 2 ml of DNPH and different volumes of 1 N NaOH soln. ranged from (2 – 6 ml). It was found that 5 ml of 1 N NaOH soln. was sufficient to give maximum absorbance (Fig. 10).

![Fig. (10): Effect of 1N NaOH volume on the absorbance of the reaction product of entacapone (3 µg/ml) with 0.1% 2,4-DNPH at 514 nm.](image)
3.2.2.3. Effect of Temperature and Heating Time

The optimum heating time was studied by heating the reaction mixture in a water bath at 80 – 85 °C for different time intervals (5 - 50 min), using (3 µg /ml) of entacapone, 2 ml of 0.1 % DNPH and 5 ml of 1 N NaOH declared that, a heating temperature of 80 – 85 °C (in a water bath) for 10 minutes was sufficient to give maximum absorbance (Fig. 11).

![Figure 11](image-url)

Fig. (11): Effect of heating time on the absorbance of the reaction product of entacapone (3 µg/ ml ) with 0.1% 2,4-DNPH at 514 nm.

3.2.2.4. Effect of time on color stability: The procedure described under (Effect of 0.1% DNPH Volume) was repeated and the stability of the formed color was studied by measuring the absorbance at the relevant maximum at different time intervals (0 - 60 min). It was found that the color was stable for at least one hour (Fig. 12).

![Figure 12](image-url)

Fig. (12): Effect of time on color stability of entacapone reaction product (3 µg/ ml ) with 0.1% 2,4-DNPH at 514 nm.
3.2.3. Method III

3.2.3.1. Effect of volume of ammonia buffer: A definite concentration of the drug was transferred to a series of 25 ml volumetric flask, followed by different volumes of ammonia buffer (PH =10) ranged from 0.05 – 0.5 ml, 2 ml of 0.3% 4-AAP then 2 ml of 1% potassium ferricyanide soln. The volume was adjusted by water and mixed well. It was found that 0.15 ml of ammonia buffer was sufficient to give maximum absorbance (Fig. 13).

![Graph](image1)

**Fig. (13):** Effect of ammonia buffer volume (PH=10) on the absorbance of entacapone reaction product (18 µg/ ml) with 0.3% 4-AAP at 493 nm.

3.2.3.2. Effect of volume of 0.3% 4-AAP: The procedure under (Effect of volume of ammonia buffer) was repeated using different volumes of 0.3% 4-AAP soln. ranged from (0.5 – 5 ml). It was found that 1.5 ml of 0.3% 4-AAP soln. was sufficient to give maximum absorbance (Fig. 14).

![Graph](image2)

**Fig. (14):** Effect of 0.3% 4-AAP volume on the absorbance of its reaction product with entacapone (18 µg/ ml) at 493 nm.
3.2.3.3. Effect of volume of 1% potassium ferricyanide: The procedure under (Effect of volume of ammonia buffer) was repeated using 2 ml of 0.3% 4-AAP soln. and different volumes of 1% potassium ferricyanide ranged from (0.5 – 5 ml). It was found that 1.5 ml of 0.3% 4-AAP soln. was sufficient to give maximum absorbance (Fig. 15).

Fig. (15): Effect of 1% potassium ferricyanide volume on the absorbance of entacapone reaction product (18 µg/ ml) with 4-AAP at 493 nm.

3.2.3.4. Effect of time on color development and stability: The procedure described under (Effect of volume of ammonia buffer) was repeated and the development and stability of the formed color was studied by measuring the absorbance at the relevant maximum at different time intervals (0 - 70 min). It was found that 5 minutes were sufficient to reach maximum absorbance and the color was stable for at least one hour (Fig. 16).

Fig. (16): Effect of time on color development and stability of entacapone reaction product (18 µg/ ml) with 4-AAP at 493 nm.
3.2.4. Method IV

3.2.4.1. Effect of Red Tetrazolium solution volume

A definite concentration of the drug solution was transferred into 10 ml volumetric flasks followed by different volumes of red tetrazolium 0.5 % solution (0.5 ml – 5 ml), mixed well, then 0.5 ml of 0.1N potassium hydroxide solution was added, the volumes completed with absolute ethanol, mixed well and the solution allowed to stand in the dark at room temperature for one hour. It was revealed that 3.5 ml of 0.5% red tetrazolium was sufficient to give a maximum absorbance (Fig. 17).

![Graph](image1)

**Fig. (17):** Effect of 0.5% red tetrazolium volume on the absorbance of its reaction product with entacapone (13 µg/ml) at 486 nm.

![Graph](image2)

**Fig. (18):** Effect of 0.1N KOH volume on the absorbance of 0.5% red tetrazolium reaction product with entacapone (13 µg/ml) at 486 nm.
3.2.4.2. Effect of potassium hydroxide volume

The procedure under (Effect of red tetrazolium solution volume) was repeated using 3.5 ml of 0.5% red tetrazolium solution and different volumes of 0.1 N potassium hydroxide solution (0.25 - 2 ml). It was revealed that 0.75 ml of 0.1N potassium hydroxide was sufficient to give the maximum absorbance (Fig. 18).

3.2.4.3. Effect of Time on Colour Development and Stability

The above procedure under (Effect of red tetrazolium solution volume) was repeated using 3.5 ml of 0.5% reagent solution and 0.75 ml of 0.1 N potassium hydroxide solution. The absorbance was measured at 10 minutes time intervals up to two hours. It was revealed that the color development needs 40 minutes to reach the maximum intensity and still stable for another one hour (Fig. 19).

Fig. (19): Effect of time on colour development and stability of 0.5% red tetrazolium reaction product with entacapone (13 µg/ml) at 486 nm.

3.3. Application to pharmaceutical preparation

Ten Parkicapone ® 200 mg Tablets were accurately weighed and finely powdered, then a quantity equivalent to 10 mg of entacapone was shaken three times with 25 ml methanol for 15 minutes then filtered into 100 ml volumetric flask and the volume was adjusted to the mark with water to obtain a concentration of (100 µg/ml). The solution was analyzed using the procedure described under method I,II,III and IV.
4. RESULTS AND DISCUSSION

4.1. Method I: The color formation by FCR with entacapone may be explained in the following manner. The mixed acids in FCR involves the following chemical species.

$$3 \text{H}_2\text{O} \cdot \text{P}_2\text{O}_5 \cdot 13\text{WO}_3 \cdot 5\text{MoO}_3 \cdot 10\text{H}_2\text{O}$$

$$3 \text{H}_2\text{O} \cdot \text{P}_2\text{O}_5 \cdot 14\text{WO}_3 \cdot 4\text{MoO}_3 \cdot 10\text{H}_2\text{O}$$

The drug affect reduction of 1, 2 or 3 oxygen atoms from tungstate and / or molybdate in the reagent producing one or more of the possible reduced species which have characteristic intense blue color.\(^{[16]}\) The color was measured against reagent blank at 758 nm and Beer’s law is obeyed in the concentration range of 1 to 8 μg/ml. The colored species was stable for more than one hour. The amount of entacapone was computed from its calibration curve. The yielded statistical results are summarized in Table (1).

Table (1): Linearity studies and regression equations of the proposed methods.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method I</th>
<th>Method II</th>
<th>Method III</th>
<th>Method IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\lambda_{\text{max}}) (nm)</td>
<td>758</td>
<td>514</td>
<td>493</td>
<td>486</td>
</tr>
<tr>
<td>Calibration range</td>
<td>(1-8 μg /ml)</td>
<td>(0.5-4.5 μg /ml)</td>
<td>(2.26 μg /ml)</td>
<td>(1-17 μg /ml)</td>
</tr>
<tr>
<td>Slope</td>
<td>0.1257</td>
<td>0.2257</td>
<td>0.0380</td>
<td>0.0593</td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.0023</td>
<td>0.0058</td>
<td>0.0066</td>
<td>0.0001</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9998</td>
<td>0.9998</td>
<td>0.9997</td>
<td>0.9999</td>
</tr>
<tr>
<td>LOD (μg/mL)</td>
<td>0.0317</td>
<td>0.0179</td>
<td>0.1628</td>
<td>0.2514</td>
</tr>
<tr>
<td>LOQ (μg/mL)</td>
<td>0.0960</td>
<td>0.0544</td>
<td>0.4934</td>
<td>0.7618</td>
</tr>
</tbody>
</table>

4.2. Method II

When a definite concentrations of entacapone soln. were heated with 2,4-DNPH soln. in 10% methanolic HCl, hydrazone may be formed after alkalinization with 1N NaOH.\(^{[17]}\) The produced hydrazone is due to the presence of entacapone ketonic group. The formed hydrazone exhibited absorption maximum at 514 nm. Good linearity was obtained in the concentration range from 0.5 to 4.5 μg/ml. The colored species was stable for more than one hour. The concentration of entacapone was calculated from the calibration curve. The yielded statistical results are summarized in Table (1).

4.3. Method III

It has been reported that 4-AAP upon oxidation with alkaline potassium ferricyanide loses 2 protons forming a nucleophilic intermediate.\(^{[18]}\) It is suggested that the intermediate undergoes nucleophilic substitution with the phenolic moieties of the drugs to produce quinoid structure producing red color which exhibit \(\lambda_{\text{max}} = 493\) nm. Beer’s law is obeyed in
the concentration range of 2 to 26 μg/ml. The colored species was stable for more than one hour. The amount of entacapone was calculated from its calibration curve. The yielded statistical results are summarized in Table (1).

4.4. Method IV: The method depends on quantitative reduction of Red Tetrazolium reagent by entacapone which transformed into a highly red colored formazan derivative. This reaction was generally enhanced in the presence of strong basic medium. The absorbance of the developed color was measured against reagent blank at 486 nm. Good linearity was obtained in the concentration range from 1 to 17 μg/ml. The colored species was stable for more than one hour. The concentration of entacapone was calculated from the calibration curve. The yielded statistical results are summarized in Table (1).

Table 2: Method validation obtained by applying the proposed methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Conc. (μg.ml⁻¹)</th>
<th>Intraday</th>
<th>Interday</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Accuracy (R%) ±SD</td>
<td>Precision (RSD%)</td>
<td>Accuracy (R%) ±SD</td>
</tr>
<tr>
<td>Method I</td>
<td>2</td>
<td>99.70 ± 0.608</td>
<td>0.610</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>100.99 ± 0.243</td>
<td>0.241</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>100.71 ± 0.430</td>
<td>0.427</td>
</tr>
<tr>
<td>Method II</td>
<td>2</td>
<td>99.82 ± 0.338</td>
<td>0.339</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>98.65 ± 0.226</td>
<td>0.229</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>99.69 ± 0.461</td>
<td>0.463</td>
</tr>
<tr>
<td>Method III</td>
<td>6</td>
<td>100.13 ± 0.438</td>
<td>0.438</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>99.58 ± 0.391</td>
<td>0.393</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>100.56 ± 0.614</td>
<td>0.610</td>
</tr>
<tr>
<td>Method IV</td>
<td>5</td>
<td>99.39 ± 0.849</td>
<td>0.854</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>100.02 ± 0.390</td>
<td>0.390</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>99.46 ± 0.455</td>
<td>0.457</td>
</tr>
</tbody>
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Table (3): Application of standard addition technique to the analysis of Parkicapone® tablets by applying the proposed methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>Amount of Entacapone (µg.mL⁻¹)</th>
<th>Standard found</th>
<th>Recovery (%)</th>
<th>Mean ± RSD%</th>
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<tbody>
<tr>
<td>I</td>
<td>Tablet Added Standard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 2</td>
<td>1.973</td>
<td>98.65</td>
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</tr>
<tr>
<td></td>
<td>2 3</td>
<td>3.048</td>
<td>101.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 4</td>
<td>3.951</td>
<td>98.78</td>
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<td></td>
<td>2 5</td>
<td>5.011</td>
<td>100.22</td>
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</tr>
<tr>
<td>II</td>
<td>2 1.5</td>
<td>1.518</td>
<td>101.20</td>
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<td></td>
<td>2 2.5</td>
<td>1.972</td>
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</tr>
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<td></td>
<td>2 3.5</td>
<td>2.528</td>
<td>101.12</td>
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</tr>
<tr>
<td>III</td>
<td>4 2</td>
<td>2.027</td>
<td>101.35</td>
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<td>6.06</td>
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<td>4 10</td>
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<td></td>
<td>4 18</td>
<td>18.09</td>
<td>100.50</td>
<td></td>
</tr>
<tr>
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<td>8 4</td>
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<td>99.97</td>
<td></td>
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<tr>
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<td>8 8</td>
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<td>8 12</td>
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<td></td>
<td>8 16</td>
<td>15.884</td>
<td>99.28</td>
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Table (4): Statistical comparison between the results obtained by applying the proposed methods and reported method for determination of entacapone in Parkicapone® tablets.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method I</th>
<th>Method II</th>
<th>Method III</th>
<th>Method IV</th>
<th>Reported Method[13]***</th>
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<tr>
<td>Mean</td>
<td>98.95</td>
<td>99.31</td>
<td>99.45</td>
<td>99.79</td>
<td>98.99</td>
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<td>S.D.</td>
<td>0.755</td>
<td>0.654</td>
<td>0.827</td>
<td>0.718</td>
<td>0.409</td>
</tr>
<tr>
<td>N*</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Variance</td>
<td>0.571</td>
<td>0.427</td>
<td>0.685</td>
<td>0.515</td>
<td>0.167</td>
</tr>
<tr>
<td>t-test**</td>
<td>0.097 (2.365)</td>
<td>0.901 (2.365)</td>
<td>1.074 (2.365)</td>
<td>2.088 (2.365)</td>
<td>-</td>
</tr>
<tr>
<td>F-value**</td>
<td>3.418 (9.117)</td>
<td>2.558 (9.117)</td>
<td>4.101 (9.117)</td>
<td>3.084 (9.117)</td>
<td>-</td>
</tr>
</tbody>
</table>

* No. of experimental.

** The values in the parenthesis are the corresponding theoretical values of t and F at (P = 0.05).

*** Determination of Entacapone using ferric chloride and 2, 2’ Bipyridine.[13]

5. Methods validation: The proposed methods were validated in compliance with the ICH guidelines.[20] Table (2) shows the accuracy and precision of the proposed methods. LOD, LOQ, linearity and range were shown earlier in Table (1).

The validity of the proposed procedures is further assessed by applying the standard addition technique showing no excipients interference. The results obtained were shown in Table (3).
6. **Statistical analysis:** Statistical comparison of the results obtained by the proposed methods and official method\(^{[13]}\) was shown in Table (4). The calculated t and F values were less than the theoretical ones indicating that there was no significant difference between the proposed and the official method with respect to accuracy and precision.

7. **CONCLUSION**

The present study deals with the development and validation of four spectrophotometric methods for the determination of entacapone using FCR (method I), 2,4-DNPH (method II), 4-AAP (method III) and Red Tetrazolium (method IV) as analytical reagents. The proposed methods offer the advantages of instrumental simplicity and high sensitivity. These methods showed satisfactory accuracy and precision. The results of recovery study proved that the methods are suitable for the determination of entacapone in tablet formulations.

8. **REFERENCES**

1. K.E. Lyons, R. Pahwa; Conversion from sustained release carbidopa/levodopa to carbidopa/levodopa/entacapone (Stalevo) in Parkinson disease patients. Clinical Neuropharmacology, 2006; 29: 73-76.


