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METHOD DEVELOPMENT AND VALIDATION OF CARBIDOPA BY USING RP-HPLC METHOD IN PHARMCEUTICAL FORMULATION

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ABSTRACT

A rapid and sensitive RP-HPLC method with UV detection (220 nm) for routine analysis of carbidopa in a pharmaceutical formulation was developed. Chromatography was performed with a mobile phase containing a methanol of assay (95.5%) with flow rate of 1.1 ml/min. Quantitation was accomplished with an internal standard method. The procedure was validated for linearity (correlation coefficient = 0.990), accuracy and limit of detection (LOD) intraday precision. To test validation of the carbidopa three factors were considered as linearity, precision, LOD where mobile phase, flow rate and pressure are respectively selected as methanol, 1.1 ml/min, 1600 pascals. For intraday precision measure the variables considered were: analyst, equipment. The RSD value (0.25%) indicated a good precision of the analytical method. The proposed method was simple; highly sensitive, precise, accurate and retention time less than 3 min indicating that the method is useful for routine quality control.

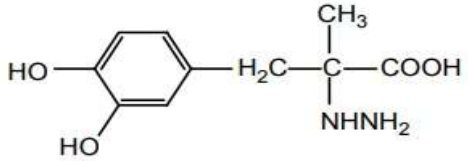
INTRODUCTION

Description: Carbidopa (Lodosyn) is a drug given to people with Parkinson's disease in order to inhibit peripheral metabolism of levodopa. This property is significant in that it allows a greater proportion of administered levodopa to cross the blood–brain barrier for central nervous system effect, instead of being peripherally metabolised into substances unable to cross said barrier.

Drug Name: Carbidopa

Brand Name: SINEMET

Structure:



IUPAC Name: 3-(3,4-Di-hydroxyphenyl)-2-hydrazinyl-2-methylpropanoic acid .

Molecular Formula: $C_{10}H_{14}N_2O_4 \cdot H_2O$.

Molecular Weight: 226.229 g/mol

Boiling Point: 528.7±50.0 °C at 760 mmHg

Category: decarboxylase inhibitors.

Solubility: Soluble in ethanol, methanol and water.

Mechanism of Action: Carbidopa is an inhibitor of the DDC which in order, inhibits the peripheral metabolism of levodopa. DDC is very important in the biosynthesis of L-tryptophan to serotonin and the modification of L-DOPA to dopamine. DDC can be found in the body periphery and in the blood-brain barrier. The action of Carbidopa is focused on peripheral DDC as this drug cannot cross the blood-brain barrier.[8] Hence, it will prevent the metabolism of levodopa in the periphery but it will not have any activity on the generation of dopamine in the brain.

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MATERIALS AND METHODS:

Instrument: The liquid chromatographic system consisted of Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD10AVP and rheadyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Interrail ODS Ultra sphere 5 µm or equivalent ODS C-18 column with 4.6 mm x 15cm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing purpose. Methanol of HPLC Grade was purchased from E-Merck, Mumbai, India. Lc grader water was obtained by double distillation and purification through Milli-Q water purification system.

The Mobile Phase: A mixture of Methanol:Acetonitrile in the ratio of 70: 30 v/v was prepared and used as mobile phase.

Preparation of mobile Phase: Accurately measured 30 ml of Acetonitrile and 70 ml of methanol were mixed by using sonication for 5 min. Diluent preparation: The mobile phase was used as the diluent. Blank Preparation: Place unused swab in 10 ml of solvent. Sonicate for 5 minutes. Squeeze swab out well. Filter through a 0.45 µm filter.

Preparation of Standard solution (Stock Solution) A stock solution of Carbidopa was prepared by Accurately weighing 50 mg of Carbidopa transferring to a 100 ml volumetric flask, dissolving in 50 ml of solvent and sonicate for 15 minutes, cool and make up to volume with solvent. Appropriate aliquot of this solution was further diluted with solvent to 100 ml with solvent. Filter through a 0.45 µm filter. From stock solution below, a series of standard solutions were prepared. Seven solutions containing 1000µg/ml, 900µg/ml, 800µg/ml, 700µg/ml, 600µg/ml and 500µg/ml of Carbidopa, relative to the working concentrations, were prepared and injected according to the method of analysis. A linear regression curve was constructed.

Preparation of sample solution Accurately weigh 10 tablets crush in motor and pestle and transfer equivalent to 150mg of Carbidopa sample into a 100 ml clean dry volumetric flask add about 50 ml of diluent and sonicate it up to 30 min to dissolve it completely and make up to the mark with the same solvent. Then it is filtered through 0.45-micron injection filter. Further pipette 2.5 ml of the above stock solution into a 25 ml volumetric flask and dilute up to the mark with diluent. Further pipette 3 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

Procedure - Inject 10µl of the standard solution, sample into the chromatographic system and measure the areas for Carbidopa peaks and calculate the % assay by using the formulae.

Linearity: From the prepared stock solution, a series of calibration standards were prepared at concentrations of 1000, 900, 800, 700, 600 and 500µg/ml using mobile phase as solvent. Each of these drug solutions (20µl) was injected into the column, the peak area and retention times were recorded. The calibration curve for Carbidopa was constructed by plotting the mean peak area against the drug concentration. Regression equation was found to be $y = 9.346x + 145.33$. ($r^2 = 0.9953$).

Precision: Accurately weigh 200 mg of Carbidopa reference standard into a 50 ml volumetric flask. Add 20 ml of solvent and sonicate for 15 minutes, cool and make up to volume with solvent. (Solution 1 to be used for sample preparation). Dilute 10 µl to 10 ml with solvent. Filter through a 0.45 µm filter. Place 10 µl of solution 1 onto a specific surface area of stainless-steel plate. Swab the surface area; take the swab stick and place into a 10 ml volumetric flask. Add 10ml of solvent and sonicate for 10 minutes. Filter through a 0.45 µm filter. The % recovery should be greater than or equal to 65%. Four replicate analysis of 20µg/ml stock solution of Carbidopa was analyzed. The % RSD was found to be 0.25 for intraday precision. The % RSD was found to be less than 2 hence the method was found to be precised**.**

RESULTS AND DISCUSSION:

Formulation: The sample solution prepared at a concentration of 100µg/ml was analysed in the developed method conditions. The method can successfully separate and identify the Carbidopa. Hencethe method was found to be suitable for routine analysis of Carbidopa and formulations.

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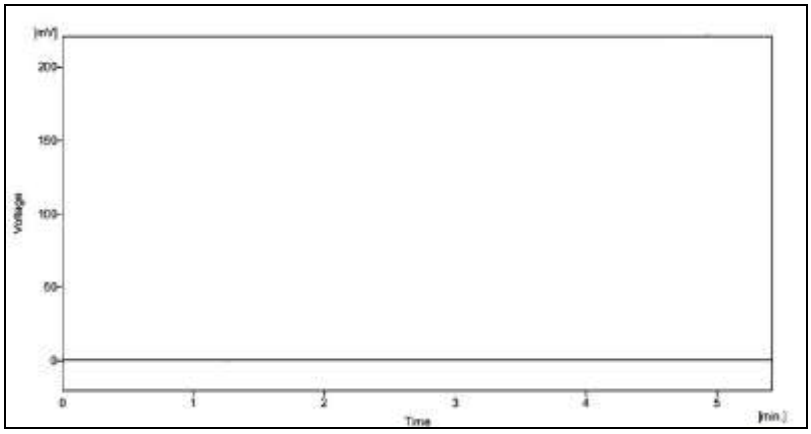
Fig.02 - wavelength scanning in UV-Visible spectrophotometer

Chromatogram for Blank:



LINEARITY:

level	Concentration(**µg/ml)**	**peak area**
Level – 1	1000 µg/ml	9578
Level – 2	900µg/ml	8589
Level – 3	800µg/ml	7572
Level – 4	700µg/ml	6493
Level – 5	600µg/ml	5730
Level - 6	500µg/ml	4967

CALIBRATION CURVE:

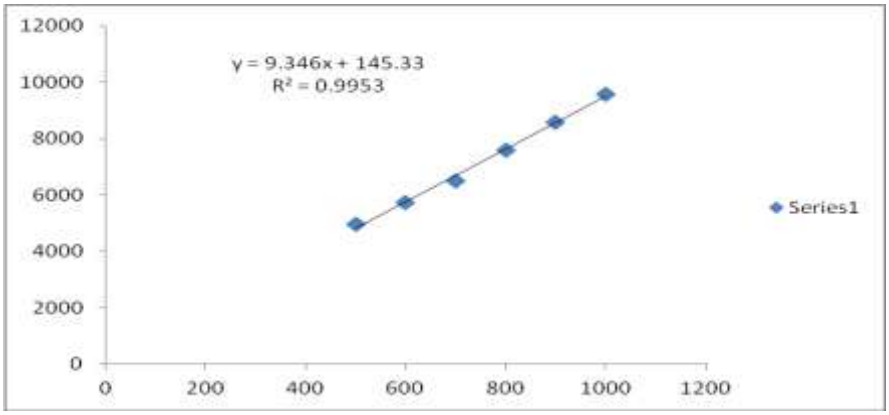


FIG.03 – Calibration curve

PRECISION:

S.NO	**Injection**	**Area value**
1	Injection 1	6493
2	Injection 2	6493
3	Injection 3	6493
4	Injection 4	6493
AVERAGE		6493
% RSD		0.25

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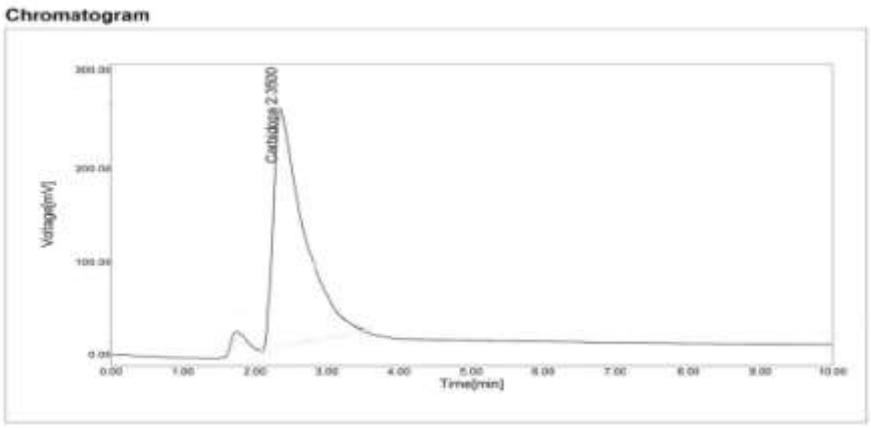

Fig.04 – Formulation results

Sample chromatogram was given. The estimation of Carbidopa was done by RP-HPLC. The assay of Carbidopa was performed with tablets and the % assay was found to be 95.5% which shows that the method is useful for routine analysis. The linearity of Carbidopa was found to be linear with a correlation coefficient of 0.991 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.25, Carbidopa which shows that the method is precise. The accuracy limit is the percentage recovery should be in the range of 90% - 103.0%. The total recovery was found to be 95.5% for Carbidopa. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD is 3 .The LOD for Carbidopa was found to be 1.5. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions**.**

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