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# METHOD DEVELOPMENT AND VALIDATION FOR ANALYSIS OF CARBIDOPA IN BULK DRUG AND FORMULATIONS.BY RP-HPLC

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## ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Carbidopa in tablet dosage form. Isocratic elution at a flow rate of 1.0ml/min was employed on a symmetry C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of methanol: $KH_2PO_4$: 75:25 (V/V). The UV detection wavelength was 262 nm and 20µl sample was injected. The retention time for Carbidopa was 1.48 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Carbidopa in tablet dosage form and bulk drug.

**Key Words:** Carbidopa, RP-HPLC, UV detection, recovery, precise, 262 nm

## INTRODUCTION:

Molecular formula $C_{10}H_{14}N_2O_4$, IUPAC Name of Carbidopa is (2*S*)-3-(3,4-dihydroxy phenyl)-2-hydrazinyl-2- Methylpropanoic acid. Weight of Carbidopa is 226.2292. Dopamine itself did not enter the brain,

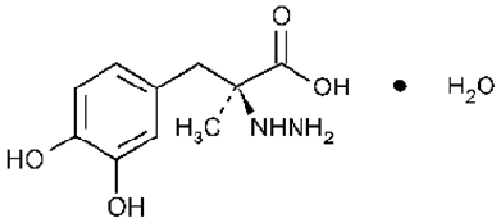


**Fig: 1** Chemical structure

## MATERIALS AND METHODS

### Chemicals and reagents

HPLC grade acetonitrile, water and sodium dihydrogen orthophosphate was purchased from Merck Specialities Pvt. Ltd. (Mumbai, India).

### Instrumentation and analytical conditions

The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase C8 column (250x4.6mm; 5µm), a 2695 binary pump, a 10µl injection loop and a 2487 dual absorbance detector and running on Waters Empower software. Isocratic elution with buffer: Methanol (25:75 v/v, pH 3.0 adjusted with orthophosphoric acid) was used at a flow rate of 1.0 mL/min. The mobile phase was prepared freshly and degassed by sonicating for 5 min before use. The UV spectrum of Carbidopa was taken using a Spectran UV-Visible spectrophotometer

### Stock and working standard solutions

Accurately weigh and transfer 10 mg of Carbidopa working standard into a 100 mL volumetric flask, add about 70 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter.

### Assay of Carbidopa tablets

Weigh 20 Carbidopa(TIDOMET) **t**ablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Carbidopa into a 100 mL volumetric flask. Add about 70 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 1 mL of the above stock solution into a 10 mL

volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter. An aliquot of this solution was injected into HPLC system. Peak area of Carbidopa was measured for the determination. The relevant results are furnished in Table-5.

**Validation procedure**

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, stability and system suitability. Standard plots were constructed with five concentrations in the range of 5-25 µg/mL prepared in triplicates to test linearity. The peak area of Carbidopa was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared Carbidopa test solution in the same equipment at a concentration value of 100 % (10 µg/mL) of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area of Carbidopa was determined and precision was reported as % RSD.

Method accuracy was tested (% recovery and % RSD of individual measurements) by analyzing sample of Carbidopa at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of Carbidopa recovered in the samples. Sample solution short term stability was tested at ambient temperature (20±10$^0$C) for three days. In order to confirm the stability of both standard solutions at 100 % level and tablet sample solutions, both solutions protected from light were re-injected after 24 and 48 hrs at ambient temperature and compared with freshly prepared solutions.

## RESULTS AND DISCUSSION

Selection of the detection wavelength**:** The UV spectra of Carbidopa in 25:75 v/v mixture of buffer and Methanol was scanned in the region between 200 and 400 nm and shows λmax at 262 nm Optimization of the chromatographic conditions**:** Proper selection of the stationary phase depends up on the nature of the sample, molecular weight and solubility. The drug Carbidopa is non-polar. Non-polar compounds preferably analyzed by reverse phase columns. Among C8 and C18, C8 column was selected. Non-polar compound is very attractive with reverse phase columns. So the elution of the

compound from the column was influenced by polar mobile phase. Mixture of phosphate buffer and acetonitrile was selected as mobile phase and the effect of composition of mobile phase on the retention time of Carbidopa was thoroughly investigated. The concentration of acetonitrile and buffer were optimized to give symmetric peak with short run time (Fig. 2). A short run time and the stability of peak asymmetry were observed in the ratio of 25:75 % v/v of $KH_2PO_4$ buffer and Methanol. It was found to be optimum mobile phase concentration.

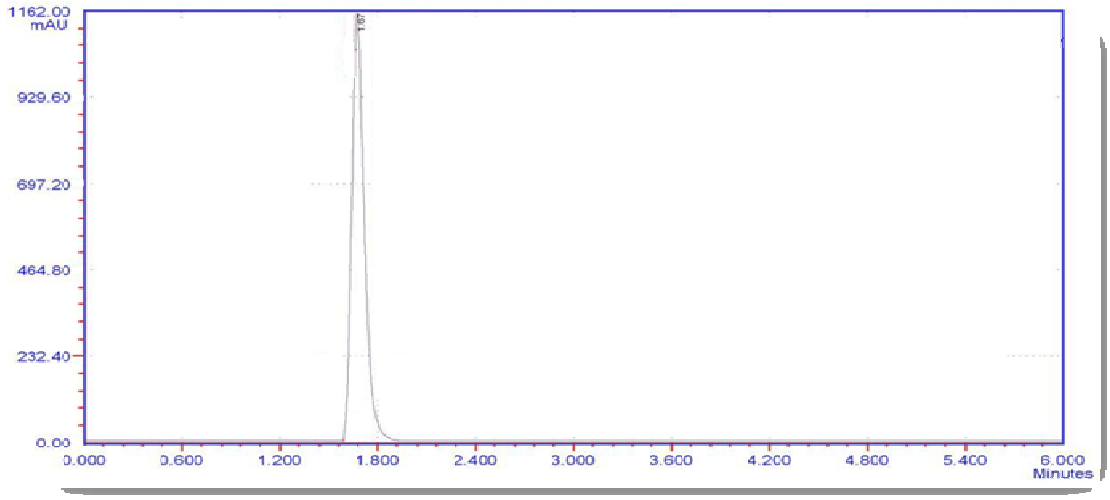


**Figure-2:** Standard chromatogram

**System suitability:** The system suitability parameter like capacity factor, asymmetry factor, tailing factor, HETP and number of theoretical plates were also calculated. It was observed that all the values are within the limits (Table 3).The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of Carbidopa in tablet formulation. The results are furnished in Table 4.

**Table-4:** Chromatographic conditions

| PARAMETERS | Values |
|---|---|
| WAVE LENGTH | 262 nm |
| BEER'S LAW | 5-25 ppm |
| C.C | 0.996 |
| RETENTION TIME | 1.48 |
| THEORETICAL PLATES | 8563 |
| TAILING FACTOR | 0.93 |
| L.O.D | 0.06 ppm |
| L.O.Q | 0.15 ppm |
| FLOW RATE | 1.0 min/ml |

**Table.5:** Market samples assay results

| SAMPLE TYPE | SAQMPLE CONC | AMOUNT FOUOND | % AMOUNT FOUND |
|---|---|---|---|
| Formulation | 20 ppm | 19.77 | 98.85 |
| Bulk drug | 20 ppm | 19.83 | 99.15 |

**Validation of method**

**Precision:** The validated method was applied for the assay of commercial tablets containing Carbidopa. Sample was analyzed for six times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results presented in good agreement with the labeled content. Assay results, expressed as the percentage of label claim, and was found to be 101.14 showing that the content of Carbidopa in tablet formulations confirmed to the content of requirements (95 – 105 %) of the label claim. Low values of standard deviation denoted very good repeatability of the measurement. Thus it was showing that the equipment used for the study was correctly and hence the developed analytical method is highly repetitive. For the intermediate precision a study carried out by the same analyst working on the same day on three consecutive days (n=3) indicated a RSD of 0.377. This indicates good method precision.

**Linearity:** Five points calibration graphs was constructed covering a concentration range 5-25 µg/mL. Linear relationships between the peak area signal of Carbidopa the corresponding drug concentration was observed. The standard deviation of the slope and intercept were low. The statistical analysis of calibration is shown in Table 1.

**Table-1:** Linearity Results

| Serial no | Concentration (µg/ml) | Area |
|---|---|---|
| 1 | 5 | 13287.4 |
| 2 | 10 | 22197.7 |
| 3 | 15 | 35501.3 |
| 4 | 20 | 44506.4 |
| 5 | 25 | 53117.4 |
| | Correlation coefficient | 0.9969 |

**Accuracy:** The data for accuracy were expressed in terms of percentage recoveries of Carbidopa in the real samples. The mean recovery data of Carbidopa in real sample were within the range of 98.3 and 101.4 %. The mean % RSD was 99.4% satisfying the acceptance criteria for the study. It was proved that there is no interference due to excipients used in tablet formulation. Hence the accuracy of the method was confirmed.

**Table-3:** Accuracy results

| %Concentratin | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50 | 35879 | 5.1 | 5.085 | 99.7 | 99.66 |
| 100 | 64532 | 10.15 | 10.13 | 99.80 | |
| 150 | 12154 | 14.5 | 14.28 | 98.48 | |

**Stability:** The stability of Carbidopa in standard and sample solutions containing determined by storing the solutions at ambient temperature (20±10$^0$C). The solutions were checked in triplicate after three successive days of storage and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 48 hrs, as during this time the results did not decrease below 98 %. This denotes that Carbidopa is stable in standard and sample solutions for at least 48 hours at ambient temperature.

## CONCLUSION

A validated RP-HPLC method has been developed for the determination of Carbidopa in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 6 min allows the analysis of a large number of samples in a short period of time. Therefore, it is suitable for the routine analysis of Carbidopa in pharmaceutical dosage form.

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