World Journal of Pharmaceutical Science and Research

www.wjpsronline.com

Research Article

ISSN: 2583-6579
SJIF Impact Factor: 6.916
Year - 2026
Volume: 5; Issue: 5
Page: 476-491

# DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING METHOD FOR LEVODOPA, CARBIDOPA & ENTACAPONE IN TABLET DOSAGE FORM: BY RP- HPLC

**Muskan Jain*[1], Dr. Sulekha Mandal[2]**

[1]Research Scholar, Mahatma Gandhi College of Pharmaceutical Sciences, Jaipur, Rajasthan- 302022.

[2]Professor, Mahatma Gandhi College of Pharmaceutical Sciences, Jaipur, Rajasthan- 302022.

***Article Received: 15 March 2026*** | | ***Article Revised: 6 April 2026*** | | ***Article Accepted: 26 April 2026***

***Corresponding Author: Muskan Jain**
Research Scholar, Mahatma Gandhi College of Pharmaceutical Sciences, Jaipur, Rajasthan- 302022.
**DOI:** https://doi.org/10.5281/zenodo.19996979

**How to cite this Article:** Muskan Jain, Dr. Sulekha Mandal (2026) DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING METHOD FOR LEVODOPA, CARBIDOPA & ENTACAPONE IN TABLET DOSAGE FORM: BY RP- HPLC. World Journal of Pharmaceutical Science and Research, 5(5), 476-491.



## ABSTRACT

This research develops and validates a stability-indicating assay method for anti-Parkinson drugs in tablet form using reverse-phase HPLC. The objective is to create accurate, cost-effective analytical methods for API quantification through RP-HPLC and UV techniques. The approach involves standard and sample solution preparation, along with buffer formulation. Results show direct proportionality between analyte concentration and obtained areas, establishing linearity. The method demonstrates a suitable range, accurate recovery rates meeting acceptance criteria, precise results within limits, and robustness with no significant system suitability changes. This research provides reliable and economical analytical methods for assessing anti-parkinson drug quality and stability.

**KEYWORDS:** RP-HPLC, liquid chromatography, validation and Quantitation.

## INTRODUCTION

Levodopa, Carbidopa, and Entacapone are commonly used to treat Parkinson's disease, which is caused by the depletion of dopamine in the brain. Levodopa is converted into dopamine, while Carbidopa inhibits the peripheral metabolism of Levodopa and Entacapone inhibits the catechol-O-methyl transferase enzyme. Together, they increase the bioavailability of Levodopa in the brain and control the cardinal symptoms of Parkinson's disease. This combination therapy is available in different strengths on the market, with Stalevo® being a well-known example. Various analytical methods such as Spectrophotometric, Spectrofluorometric, TLC, and HPLC have been used to determine the concentrations of these drugs in pharmaceutical formulations. In this study, a validated stability-

indicating RP-HPLC method was proposed to determine the presence of degradation products and assess the purity and stability of these drugs in oral contraceptive tablet formulations. The proposed method is simple, accurate, rapid, reproducible, and economical, and can effectively separate all the degradants from the drug with a run time of 11.0 minutes. The results of this study will help in the formulation and characterization of degradation compounds, as well as open a new scope for the toxicity study of degraded components.

## MATERIALS AND METHODS

### Chemicals

Drugs & Impurities of [D2122] purchased from Torrent Research Centre, Ortho-Phosphoric Acid AR grade, Merck, Acetonitrile, Iso- Propyl Alcohol from HPLC grade, Rankem.

### Chromatographic conditions

The chromatographic separations were performed on a phenomenex 100 C18 (250 × 4.6 mm), 5 µm column at room temperature using flow rate of 0.8 ml/minute with run time 6.0 minutes and UV detection wavelength at 268 nm. Injection volume was set as 50 µl. Acetonitrile and water in the ration of 50:50% v/v, adjusted to pH to 3.0 ± 0.05 using ortho-phosphoric acid was used as mobile phase which was filtered (0.2 µm finer porosity nylon membrane filter) and degassed by sonication. Mobile phase is used as the diluent.

### Preparation of standard stock solution

RM standard equivalent to 100 mg was dissolved in 100 ml of diluent to prepare standard stock solution. The stock solution was diluted to get a final standard concentration of 100 µg/ml.

### Preparation of sample solution

Twenty tablets each containing 1 mg of rasagiline were weighed, average weight found, and finely powdered. The sample solution was prepared by taking weight equivalent to 10 mg RM from powdered 20 tablets into 100-ml volumetric flask and kept for sonication with 75 ml mobile phase for 30 minutes and shaken occasionally. After cooling to room temperature, the volume was made up with mobile phase, solution was filtered (0.2-µm nylon membrane filter).

### Stock Solution Preparation

Accurately weighed and transferred 100 mg of RM standard in to dry and clean 100 ml volumetric flask, dissolved and diluted to 100 ml with diluent, to get a concentration of 1,000 µg/ ml. This solution was used as a stock solution for further studies.

## RESULTS AND DISCUSSION

### 1. Specificity

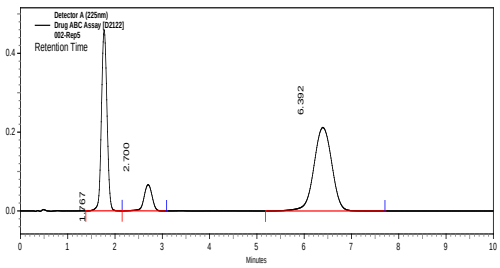


**Fig. 1: Peak Purity for the Standard.**

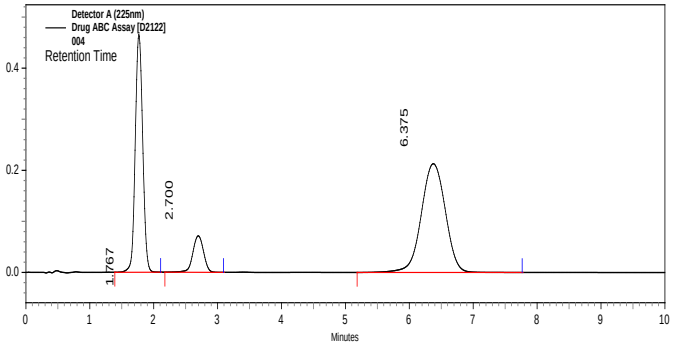


**Fig. 2: Sample Not Spike.**

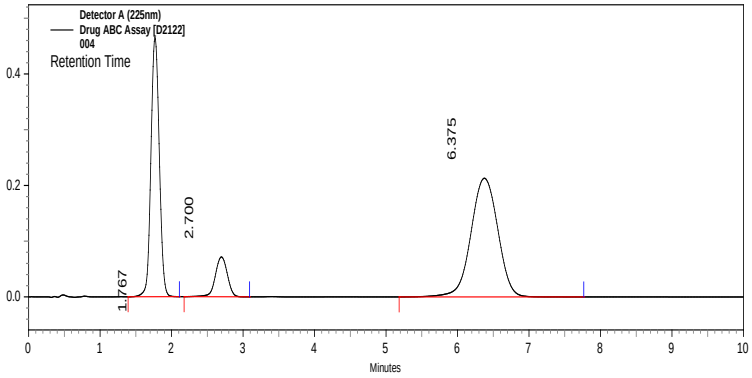


**Fig. 3: Sample Spike in HPLC.**

**Table 1: Data indicating peak purity index of Levodopa, Carbidopa & Entacopne.**

| Sample | Peak Purity Index | | |
|---|---|---|---|
| | Levodopa | Carbidopa | Entacopne |
| Standard preparation | 0.9999 | 0.9999 | 0.9999 |
| Sample preparation | 0.9999 | 0.9998 | 1.0000 |

**Interference from Degradation Products**

**Acid degradation:** Heated for 3 hour in 1N hydrochloric acid at 80°C.

**Base degradation:** Heated for 1 hour in 0.1N sodium hydroxide at 80°C.

### 6.2. Linearity and Range

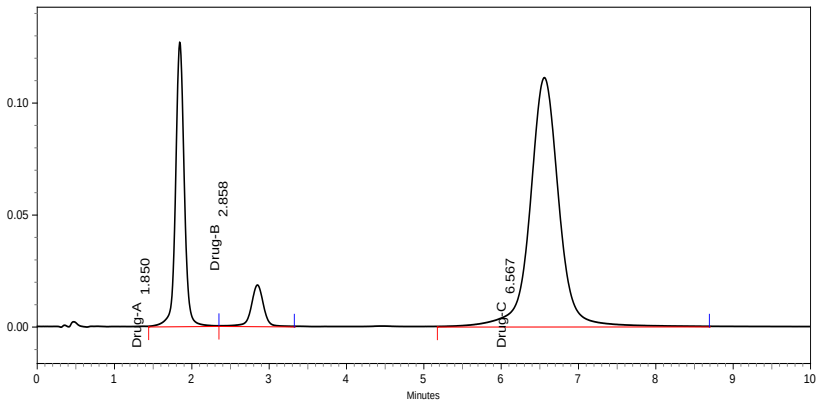


**Fig. 4: Linearity Chromatogram for Level-1.**

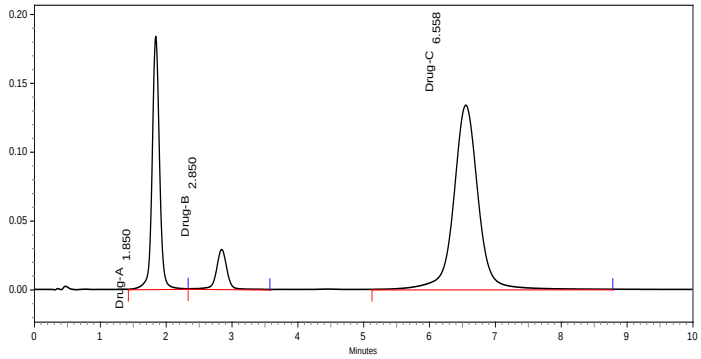


**Fig. 5: Linearity Chromatogram for Level-2.**

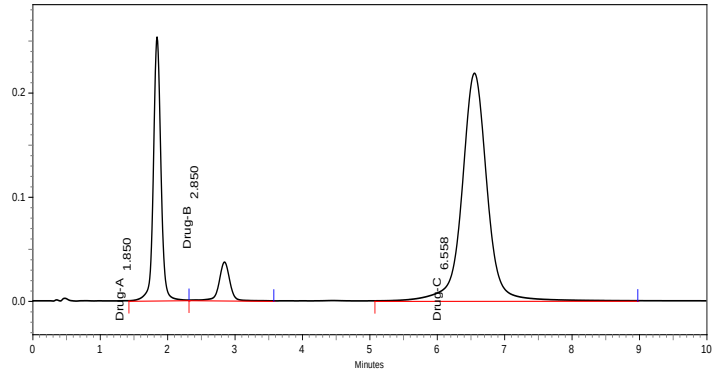


**Fig. 6: Linearity Chromatogram for Level-3.**

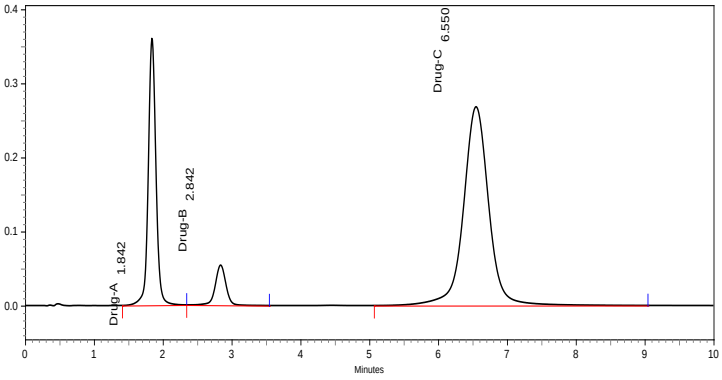


**Fig. 7: Linearity Chromatogram for Level-4.**

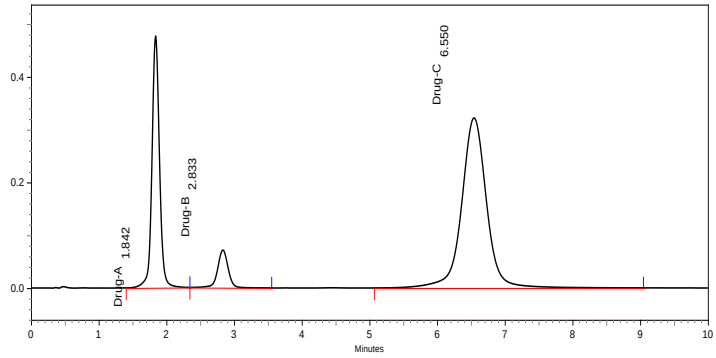


**Fig. 8: Linearity Chromatogram for Level-5.**

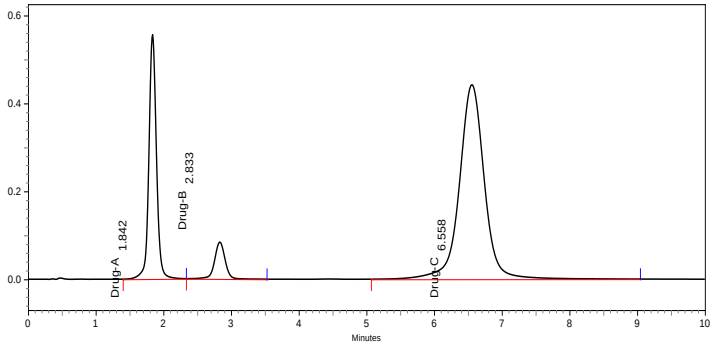


**Fig. 9: Linearity Chromatogram for Level-6.**

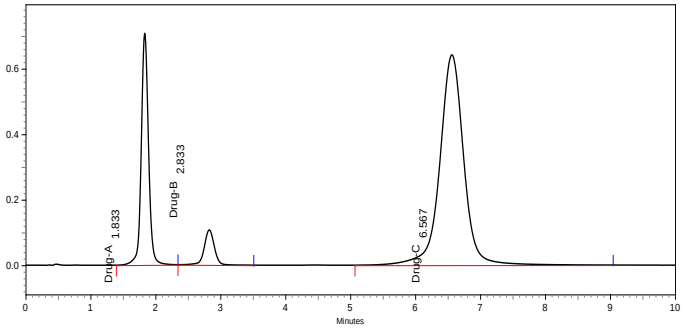


**Fig. 10: Linearity Chromatogram for Level-7.**

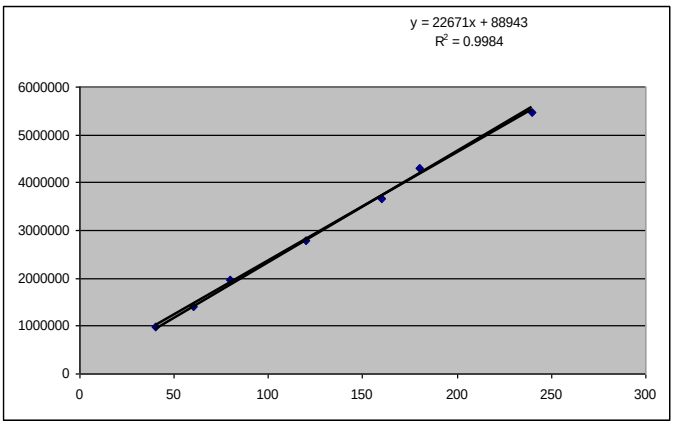


**Fig. 11: Linearity Graph of Levodopa**

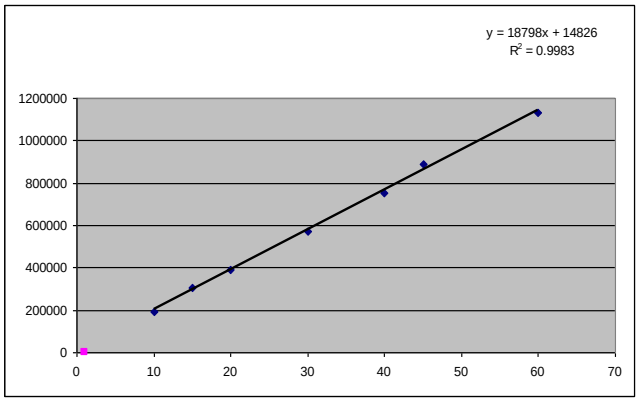


**Fig. 12: Linearity Graph of Carbidopa**

### 6.3. Accuracy (Recovery)

**Table 2: Data indicating recovery study of Levodopa.**

| Level of Recovery | Area | Mean Area | Added Amount ( µg / ml) | Recovered Amount ( µg / ml) | % Recovery | Mean % Recovery | % RSD |
|---|---|---|---|---|---|---|---|
| 25% | 1033504 | 1033504 | 39.9752 | 40.2547 | 100.7 | 101.0 | 0.69 |
| | 1044543 | 1044543 | 39.9593 | 40.6845 | 101.8 | | |
| | 1031797 | 1031797 | 39.9991 | 40.1880 | 100.5 | | |
| 75% | 3056724 | 3056724 | 119.5992 | 119.0580 | 99.5 | 100.0 | 0.61 |
| | 3068683 | 3068683 | 119.5912 | 119.5238 | 99.9 | | |
| | 3092069 | 3092069 | 119.6031 | 120.4347 | 100.7 | | |
| 150% | 6135842 | 6135842 | 238.9993 | 238.9882 | 100.0 | 100.4 | 0.69 |
| | 6134708 | 6134708 | 238.9873 | 238.9441 | 100.0 | | |
| | 6206391 | 6206391 | 238.9754 | 241.7361 | 101.2 | | |
| **Mean** | | | | | | **100.4** | **0.66** |

**Table 3: Data indicating recovery study of Carbidopa.**

| Level of Recovery | Area | Mean Area | Added Amount ( µg / ml) | Recovered Amount ( µg / ml) | % Recovery | Mean % Recovery | % RSD |
|---|---|---|---|---|---|---|---|
| 25% | 215465 | 215465 | 9.4132 | 9.3547 | 99.4 | 99.7 | 0.64 |
| | 218033 | 218033 | 9.4243 | 9.4662 | 100.4 | | |
| | 215638 | 215638 | 9.4390 | 9.3622 | 99.2 | | |
| 93.8% | 782870 | 782870 | 34.6343 | 33.9893 | 98.1 | 98.5 | 0.59 |
| | 784342 | 784342 | 34.6380 | 34.0532 | 98.3 | | |
| | 791201 | 791201 | 34.6196 | 34.3510 | 99.2 | | |
| 150% | 1263730 | 1263730 | 55.5345 | 54.8665 | 98.8 | 99.1 | 0.67 |
| | 1263435 | 1263435 | 55.5493 | 54.8537 | 98.7 | | |
| | 1277721 | 1277721 | 55.5419 | 55.4739 | 99.9 | | |
| **Mean** | | | | | | **99.1** | **0.63** |

**Table 4: Data indicating recovery study of Entacopne.**

| Level of Recovery | Area | Mean Area | Added Amount ( µg / ml) | Recovered Amount ( µg / ml) | % Recovery | Mean % Recovery | % RSD |
|---|---|---|---|---|---|---|---|
| | 3050940 | 3050940 | 79.7428 | 80.5320 | 101.0 | | |
| | 2989355 | 2989355 | 79.7388 | 78.9064 | 99.0 | | |
| 200% | 11898033 | 11898033 | 318.3428 | 314.0582 | 98.7 | 98.8 | 0.27 |
| | 11896420 | 11896420 | 318.3548 | 314.0156 | 98.6 | | |
| | 11946621 | 11946621 | 318.3587 | 315.3407 | 99.1 | | |
| 300% | 17983531 | 17983531 | 477.6097 | 474.6598 | 99.4 | 99.2 | 0.15 |
| | 17934234 | 17934234 | 477.5938 | 473.3885 | 99.1 | | |
| | 17945590 | 17945590 | 477.5898 | 473.6883 | 99.2 | | |
| **Mean** | | | | | | **99.2** | **0.52** |

### 6.4. Precision

**Table 5: Method precision data for analysis of Levodopa in tablet formulation.**

| Sample No. | Area | Mean Area | % Assay |
|---|---|---|---|
| 1 | 3630898 | 3635047 | 98.7 |
| | 3639196 | | |
| 2 | 3708169 | 3707041 | 100.7 |
| | 3705912 | | |
| 3 | 3694507 | 3697478 | 100.4 |
| | 3700449 | | |

| | | | |
|---|---|---|---|
| 4 | 3659499<br>3657315 | 3658407 | 99.4 |
| 5 | 3745183<br>3752984 | 3749084 | 101.8 |
| 6 | 3742365<br>3735834 | 3739100 | 101.5 |
| **Mean Assay** | | | **100.4** |
| **SD** | | | **1.20** |
| **% RSD** | | | **1.2** |
| **95% C.I** | | | **1.26** |

**Table 6: Method precision data for analysis of Carbidopa in tablet formulation.**

| Sample No. | Area | Mean Area | % Assay |
|---|---|---|---|
| 1 | 768723<br>768534 | 768629 | 98.9 |
| 2 | 782594<br>783887 | 783241 | 100.8 |
| 3 | 780601<br>781211 | 780906 | 100.5 |
| 4 | 773455<br>771884 | 772670 | 99.4 |
| 5 | 791271<br>792386 | 791829 | 101.9 |
| 6 | 791110<br>790458 | 790784 | 101.8 |
| **Mean Assay** | | | **100.6** |
| **SD** | | | **1.22** |
| **% RSD** | | | **1.21** |
| **95% C.I** | | | **1.28** |

**Table 7: Method precision data for analysis of Entacopne in tablet formulation.**

| Sample No. | Area | Mean Area | % Assay |
|---|---|---|---|
| 1 | 5633696<br>5658686 | 5646191 | 99.4 |
| 2 | 5631947<br>5647472 | 5639710 | 99.3 |
| 3 | 5616628<br>5602937 | 5609783 | 98.8 |
| 4 | 5580592<br>5580320 | 5580456 | 98.3 |
| 5 | 5696272<br>5709424 | 5702848 | 100.4 |
| 6 | 5760699<br>5774674 | 5767687 | 101.6 |
| **Mean Assay** | | | **99.6** |
| **SD** | | | **1.19** |
| **% RSD** | | | **1.19** |
| **95% C.I** | | | **1.25** |

### 6.4. Intermediate Precision (Ruggedness)

**7. Table 8: Intermediate precision data for analysis of Levodopa in tablet formulation.**

| Sample No. | Area | Mean Area | % Assay |
|---|---|---|---|
| 1 | 3997865<br>4007659 | 4002762 | 98.6 |
| 2 | 4000441<br>4011335 | 4005888 | 98.6 |

| | | | |
|---|---|---|---|
| 3 | 4011188<br>4025163 | 4018176 | 98.9 |
| 4 | 4015007<br>4012755 | 4013881 | 98.8 |
| 5 | 4070195<br>4075814 | 4073005 | 100.3 |
| 6 | 4058307<br>4063670 | 4060989 | 100.0 |
| **Mean Assay** | | | **99.2** |
| **SD** | | | **0.75** |
| **% RSD** | | | **0.76** |
| **95% C.I** | | | **0.79** |

**Table 9: Intermediate precision data for analysis of Carbidopa in tablet formulation.**

| Sample No. | Area | Mean Area | % Assay |
|---|---|---|---|
| 1 | 864924<br>866504 | 865714 | 99.2 |
| 2 | 864894<br>867622 | 866258 | 99.2 |
| 3 | 868270<br>870793 | 869532 | 99.6 |
| 4 | 866182<br>868262 | 867222 | 99.3 |
| 5 | 880131<br>881895 | 881013 | 100.9 |
| 6 | 877449<br>878020 | 877735 | 100.5 |
| **Mean Assay** | | | 99.8 |
| **SD** | | | 0.74 |
| **% RSD** | | | 0.74 |
| **95% C.I** | | | 0.78 |

**Table 10: Intermediate precision data for analysis of Entacapone in tablet formulation.**

| Sample No. | Area | Mean Area | % Assay |
|---|---|---|---|
| 1 | 6217047<br>6229588 | 6223318 | 99.0 |
| 2 | 6233305<br>6235583 | 6234444 | 99.2 |
| 3 | 6246657<br>6268407 | 6257532 | 99.5 |
| 4 | 6254305<br>6241487 | 6247896 | 99.4 |
| 5 | 6295488<br>6322932 | 6309210 | 100.4 |
| 6 | 6306853<br>6309544 | 6308199 | 100.3 |
| **Mean Assay** | | | 99.6 |
| **SD** | | | 0.58 |
| **% RSD** | | | 0.58 |
| **95% C.I** | | | 0.61 |

**Table 11: Comparison for Levodopa.**

| Comparison | Method Precision | Intermediate Precision |
|---|---|---|
| **Results** | **% Assay** | **% Assay** |
| **Test Sample-1** | 98.7 | 98.6 |
| **Test Sample-2** | 100.7 | 98.6 |
| **Test Sample-3** | 100.4 | 98.9 |
| **Test Sample-4** | 99.4 | 98.8 |
| **Test Sample-5** | 101.8 | 100.3 |
| **Test Sample-6** | 101.5 | 100.0 |
| **Mean Assay** | **100.4** | **99.2** |
| **Std. Dev** | 1.20 | 0.75 |
| **% RSD** | 1.20 | 0.76 |
| **95% C.I** | 1.26 | 0.79 |
| **Absolute Difference** | 1.2 | |

**Table 12: Comparison for Carbidopa.**

| Comparison | Method Precision | Intermediate Precision |
|---|---|---|
| **Results** | **% Assay** | **% Assay** |
| **Test Sample-1** | 98.9 | 99.2 |
| **Test Sample-2** | 100.8 | 99.2 |
| **Test Sample-3** | 100.5 | 99.6 |
| **Test Sample-4** | 99.4 | 99.3 |
| **Test Sample-5** | 101.9 | 100.9 |
| **Test Sample-6** | 101.8 | 100.5 |
| **Mean Assay** | **100.6** | **99.8** |
| **Std. Dev** | 1.22 | 0.74 |
| **% RSD** | 1.21 | 0.74 |
| **95% C.I** | 1.28 | 0.78 |
| **Absolute Difference** | 0.8 | |

**Table 13: Comparison for Entacapone.**

| Comparison | Method Precision | Intermediate Precision |
|---|---|---|
| **Results** | **% Assay** | **% Assay** |
| **Test Sample-1** | 99.4 | 99.0 |
| **Test Sample-2** | 99.3 | 99.2 |
| **Test Sample-3** | 98.8 | 99.5 |
| **Test Sample-4** | 98.3 | 99.4 |
| **Test Sample-5** | 100.4 | 100.4 |
| **Test Sample-6** | 101.6 | 100.3 |
| **Mean Assay** | **99.6** | **99.6** |
| **Std. Dev** | 1.19 | 0.58 |
| **% RSD** | 1.19 | 0.58 |
| **95% C.I** | 1.25 | 0.61 |
| **Absolute Difference** | 0.0 | |

### 7.4. Solution Stability.

**Table 14: Solution stability data for Levodopa.**

| Time | Standard | | | Tablet | | |
|---|---|---|---|---|---|---|
| | Area | % Assay | Absolute Difference | Area | % Assay | Absolute Difference |
| Initial | 339504 | 100.0 | - | 455807 | 99.9 | - |
| After 24 hrs | 337135 | 99.8 | 0.2 | 456820 | 100.8 | 0.9 |
| After 48 hrs | 337172 | 99.9 | 0.1 | 457863 | 101.0 | 1.1 |

**Table 15: Solution stability data for Carbidopa.**

| Time | Standard | | | Tablet | | |
|---|---|---|---|---|---|---|
| | Area | % Assay | Absolute Difference | Area | % Assay | Absolute Difference |
| Initial | 112705 | 100.0 | - | 75601 | 99.8 | - |
| After 24 hrs | 115324 | 100.0 | 0.0 | 75369 | 98.9 | 0.9 |
| After 48 hrs | 115233 | 100.0 | 0.0 | 75145 | 98.1 | 1.7 |

**Table 16: Solution stability data for Entacopne.**

| Time | Standard | | | Tablet | | |
|---|---|---|---|---|---|---|
| | Area | % Assay | Absolute Difference | Area | % Assay | Absolute Difference |
| Initial | 5211819 | 100.0 | - | 7144260 | 101.7 | - |
| After 24 hrs | 5196496 | 99.9 | 0.1 | 7140247 | 101.6 | 0.1 |
| After 48 hrs | 5181583 | 99.8 | 0.2 | 7140232 | 101.7 | 0.0 |

**7.5. Filter media interference**

**Table 17: Data indicating %assay of filtered and unfiltered sample for Levodopa.**

| Sample Preparation No. | Filtered sample | | Unfiltered sample | |
|---|---|---|---|---|
| | Average Area | Assay (%) | Average Area | Assay (%) |
| 1. | 3780492 | 100.8 | 3772845 | 100.1 |
| **Difference of % Assay** | 0.7 | | | |

**Table 18: Data indicating %assay of filtered and unfiltered sample for Carbidopa.**

| Sample Preparation No. | Filtered sample | | Unfiltered sample | |
|---|---|---|---|---|
| | Average Area | Assay (%) | Average Area | Assay (%) |
| 1. | 831264 | 99.8 | 831152 | 99.3 |
| **Difference of % Assay** | 0.5 | | | |

**Table 19: Data indicating %assay of filtered and unfiltered sample for Entacopne.**

| Sample Preparation No. | Filtered sample | | Unfiltered sample | |
|---|---|---|---|---|
| | Average Area | Assay (%) | Average Area | Assay (%) |
| 1. | 5571851 | 100.4 | 5571721 | 100.0 |
| **Difference of % Assay** | 0.4 | | | |

.

**7.6. Flow rate**

**Table 20: Change the flow rate of Mobile Phase for Levodopa.**

| Standard repetitions | 1.50 ml/min (As per Method) | 1.40 ml/min (-0.10 ml/min) | 1.60 ml/min (+0.10 ml/min) |
|---|---|---|---|
| | Drug-A Area | | |
| 1 | 4276657 | 4552222 | 4028928 |
| 2 | 4271431 | 4552115 | 4032977 |
| 3 | 4267778 | 4564956 | 4036282 |
| 4 | 4265459 | 4564179 | 4032202 |
| 5 | 4263931 | 4569294 | 4035764 |
| **Mean Area** | 4269051 | 4560553 | 4033231 |
| **% RSD** | **0.12** | **0.17** | **0.07** |

**Table 21: Change the flow rate of Mobile Phase for Carbidopa.**

| Standard repetitions | 1.5 ml/min (As per Method) | 1.40 ml/min (-0.10 ml/min) | 1.60 ml/min (+0.10 ml/min) |
|---|---|---|---|
| | Drug-B Area | | |
| 1 | 897864 | 952223 | 846375 |
| 2 | 897726 | 953289 | 847650 |
| 3 | 896565 | 954811 | 849023 |

| | | | |
|---|---|---|---|
| 4 | 895552 | 954745 | 847935 |
| 5 | 895549 | 954160 | 849080 |
| **Mean Area** | 896651 | 953846 | 848013 |
| **% RSD** | **0.13** | **0.11** | **0.13** |

**Table 22: Change the flow rate of Mobile Phase for Entacapone.**

| **Standard repetitions** | **1.5 ml/min (As per Method)** | **1.40 ml/min (-0.10 ml/min)** | **1.60 ml/min (+0.10 ml/min)** |
|---|---|---|---|
| | **Drug-C Area** | | |
| 1 | 6196727 | 6588919 | 5882834 |
| 2 | 6218601 | 6593242 | 5891462 |
| 3 | 6227159 | 6604280 | 5917696 |
| 4 | 6228634 | 6645701 | 5936534 |
| 5 | 6232111 | 6669178 | 5942173 |
| **Mean Area** | 6220646 | 6620264 | 5914140 |
| **% RSD** | **0.23** | **0.53** | **0.45** |

### 7.7. Column oven Temperature

**Table 23: Change the Column Oven Temperature for Levodopa.**

| **Standard repetitions** | **40°C(As per Method)** | **35°C (-5°C)** | **45°C (+5°C)** |
|---|---|---|---|
| | **Drug-A Area** | | |
| 1 | 4276657 | 4311851 | 4284282 |
| 2 | 4271431 | 4306754 | 4258380 |
| 3 | 4267778 | 4307879 | 4279656 |
| 4 | 4265459 | 4305355 | 4270695 |
| 5 | 4263931 | 4297510 | 4273503 |
| **Mean Area** | 4269051 | 4305870 | 4278703 |
| **% RSD** | **0.12** | **0.12** | **0.15** |

**Table 24: Change the Column Oven Temperature for Carbidopa.**

| **Sample preparation** | **40°C(As per Method)** | **35°C (-5°C)** | **45°C (+5°C)** |
|---|---|---|---|
| | **Drug-B Area** | | |
| 1 | 895999 | 905727 | 910640 |
| 2 | 896169 | 906713 | 913472 |
| **Mean Area** | 896084 | 906220 | 912056 |
| **% Assay** | **94.4** | **95.4** | **96.2** |
| **Absolute Difference** | **-** | **1.0** | **1.8** |

**Table 25: Change the Column Oven Temperature for Entacapone.**

| **Sample preparation** | **40°C(As per Method)** | **35°C (-5°C)** | **45°C (+5°C)** |
|---|---|---|---|
| | **Drug-C Area** | | |
| 1 | 6248037 | 6310802 | 6374520 |
| 2 | 6248174 | 6328485 | 6396079 |
| **Mean Area** | 6248106 | 6319646 | 6385300 |
| **% Assay** | **101.3** | **101.6** | **103.3** |
| **Absolute Difference** | **-** | **0.3** | **2.0** |

### 7.8. pH

**Table 26: Change the Mobile Phase pH for Levodopa.**

| **Standard repetitions** | **2.0 (As per Method)** | **1.8 (-0.2)** | **2.2 (+0.2)** |
|---|---|---|---|
| | **Drug-A Area** | | |
| 1 | 4276657 | 4266396 | 4248340 |
| 2 | 4271431 | 4261138 | 4233739 |
| 3 | 4267778 | 4257224 | 4232054 |
| 4 | 4265459 | 4243121 | 4219600 |

| | | | |
|---|---|---|---|
| 5 | 4263931 | 4237654 | 4210756 |
| **Mean Area** | 4269051 | 4253107 | 4228898 |
| **% RSD** | **0.12** | **0.29** | **0.34** |

**Table 27: Change the Mobile Phase pH for Carbidopa.**

| **Standard repetitions** | **2.0 (As per Method)** | **1.8 (-0.2)** | **2.2 (+0.2)** |
|---|---|---|---|
| | **Drug-B Area** | | |
| 1 | 897864 | 900888 | 886590 |
| 2 | 897726 | 899246 | 882613 |
| 3 | 896565 | 899170 | 882856 |
| 4 | 895552 | 886712 | 880074 |
| 5 | 895549 | 895277 | 879440 |
| **Mean Area** | 896651 | 896259 | 882315 |
| **% RSD** | **0.13** | **0.64** | **0.32** |

**Table 28: Change the Mobile Phase pH for Entacapone.**

| **Standard repetitions** | **2.0 (As per method)** | **1.8 (-0.2)** | **2.2 (+0.2)** |
|---|---|---|---|
| | **Drug-C Area** | | |
| 1 | 6196727 | 6212979 | 6106566 |
| 2 | 6218601 | 6186480 | 6100844 |
| 3 | 6227159 | 6181096 | 6096606 |
| 4 | 6228634 | 6202770 | 6071717 |
| 5 | 6232111 | 6197540 | 6072849 |
| **Mean Area** | 6220646 | 6196173 | 6089716 |
| **% RSD** | **0.23** | **0.21** | **0.27** |

### 7.9. System suitability

**Table 29: Mean values of system suitability parameters for Levodopa.**

| **Sr. No.** | **Parameters** | **Levodopa** |
|---|---|---|
| 1. | Peak area | 4269051 |
| 2. | No. of theoretical plates | 1569 |
| 3. | Retention time (min) | 1.88 |
| 4. | Asymmetry/USP Tailing | 1.18 |
| 5. | % RSD | 0.12 |

**Table 30: Mean values of system suitability parameters for Carbidopa.**

| **Sr. No.** | **Parameters** | **Carbidopa** |
|---|---|---|
| 1. | Peak area | 896651 |
| 2. | No. of theoretical plates | 1996 |
| 3. | Retention time (min) | 2.85 |
| 4. | Asymmetry/USP Tailing | 1.16 |
| 5. | % RSD | 0.13 |

**Table 31: Mean values of system suitability parameters for Entacapone.**

| **Sr. No.** | **Parameters** | **Entacapone** |
|---|---|---|
| 1. | Peak area | 6220646 |
| 2. | No. of theoretical plates | 2113 |
| 3. | Retention time (min) | 6.83 |
| 4. | Asymmetry/ USP Tailing | 1.11 |
| 5. | % RSD | 0.23 |

### 7.10. CALCULATION FOR ASSAY

% Assay= At x Ws x 100 x AW x 100 x P/ As 100 Wt LC 100

At= Avg. peak area due to [D2122] in chromatogram with sample preparation

As= Avg. peak area due to [D2122] in chromatogram with standard preparation

Ws= Weight of standard in mg

Wt= Weight of sample in mg

P= % Purity of standard as such

AW= Average weight in mg

LC= Label claim

**Table 32: Order of injection for calculation of Assay.**

| S. No. | Solution | No of injections |
|---|---|---|
| 1 | Diluent (Blank) | 1 |
| 2 | Standard Preparation | 5 |
| 3 | Sample Preparation | 2 |

**Table 33: Calculation for %Assay for Levodopa.**

| | |
|---|---|
| **Standard Weight – (mg)** | 100 |
| **Standard Potency – (mg)(%)** | 99.52 |
| **Standard Concentration (mg/ml)** | 0.16 |
| **Label Claim – (mg)** | 200 |
| **Avg. weight of Tablets (mg)** | 912 |

**Table 34: Calculation for %Assay for Carbidopa.**

| | |
|---|---|
| **Standard Weight – (mg)** | 25 |
| **Standard Potency – (mg)(%)** | 92.25 |
| **Standard Concentration (mg/ml)** | 0.04 |
| **Label Claim – (mg)** | 50 |
| **Avg. weight of Tablets (mg)** | 912 |

**Table 35: Calculation for %Assay for Entacapone.**

| | |
|---|---|
| **Standard Weight – (mg)** | 50 |
| **Standard Potency – (mg)(%)** | 99.42 |
| **Standard Concentration (mg/ml)** | 0.16 |
| **Label Claim – (mg)** | 200 |
| **Avg. weight of Tablets (mg)** | 912 |

**Table 36: Results of Assay by developed RP-HPLC method for Levodopa**

| Parameters | Levodopa |
|---|---|
| **Standard Area** | 3660809 |
| | 3660851 |
| | 3664071 |
| | 3665381 |
| | 3661961 |
| **Mean Area** | 3662615 |
| **Sample Area** | 3603512 |
| | 3610078 |
| **Mean Area** | 3606795 |
| **%Assay** | **98.4** |

**Table 37: Results of Assay by developed RP-HPLC method for Carbidopa.**

| Parameters | Carbidopa |
|---|---|
| Standard Area | 742706 |
| | 744875 |
| | 743479 |
| | 744157 |
| | 744838 |
| Mean Area | 744011 |
| Sample Area | 762754 |
| | 763359 |
| Mean Area | 763057 |
| %Assay | **95.8** |

**Table 38: Results of Assay by developed RP-HPLC method for Entacapone.**

| Parameters | Entacapone |
|---|---|
| Standard Area | 5493759 |
| | 5503133 |
| | 5500496 |
| | 5495856 |
| | 5500085 |
| Mean Area | 5498446 |
| Sample Area | 5617662 |
| | 5615083 |
| Mean Area | 5616373 |
| %Assay | **102.6** |

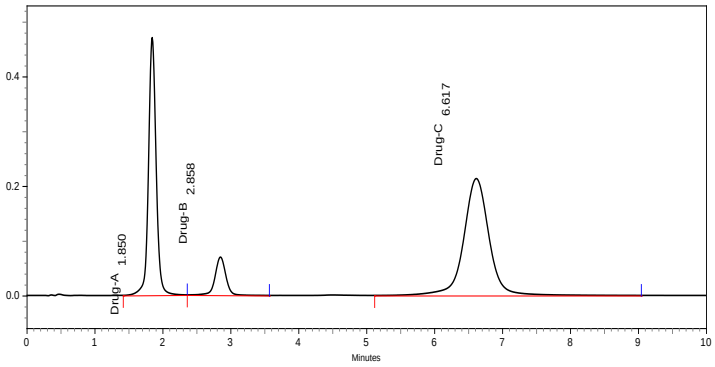


**Fig. 13: Standard Chromatogram for Assay**

**Table 39: Observation and remarks of method development for [D2122].**

| Sr.No. | Trails Taken | Observation | Remarks |
|---|---|---|---|
| 1 | Buffer : Methanol (30:70 v/v),<br>Isocratic<br>Flow rate 1.2 ml/min<br>Column:- Inertsil ODS 3, 150 mm x 4.6 mm, 5 µm | The impurities were not well separated from main peak. | Not Satisfactory |
| 2 | Mobile Phase : Buffer: ACN (95:5 v/v)<br>Isocratic<br>Flow rate 1.2 ml/min<br>Column:- Inertsil ODS 3, 50 mm x 4.6 mm, 5 µm | Resolution between two impurities was not good. | Not Satisfactory |
| 3. | Mobile Phase A: Buffer: ACN (95:5 v/v)<br>Mobile Phase B: Methanol,<br>Flow rate 1.2 ml/min<br>Column:- - Inertsil ODS 3, 50 mm x 4.6 mm, 5 µm | Main Peak eluted very early | Not Satisfactory |
| 4 | Mobile Phase A: Buffer: ACN: IPA (78:12:10v/v)<br>Isocratic<br>Flow rate 1.5 ml/min<br>Column:- Inertsil ODS 3, 50 mm x 4.6 mm, 5 µm | Peak shape was found to be good, no impurities merge with the main peak, good resolution. | Satisfactory |

**Table 40: Data indicating peak purity Factor of [D2122] and its impurities.**

| Sample | Peak purity Factor | | |
|---|---|---|---|
| | Main Peak | Impurity A | Impurity B |
| Standard preparation | 999.99 | ---- | ---- |
| Sample preparation | 999.99 | --- | ---- |
| Impurity - A | ---- | ---- | ---- |
| Impurity -B | ---- | ---- | ---- |
| Sample spiked with known impurities preparation | 999.99 | ---- | ---- |

**Table 41: System suitability parameters.**

| Parameter | | | Theoretical Plates | Tailing Factor | %RSD |
|---|---|---|---|---|---|
| Limits | | | Not less than 2000 | Not more than 2.0 | Not more than 2.0% |
| 1 | **Specificity** | | | | |
| | 1.1 | Specificity-Part-A | 2574 | 1.17 | 0.13 |
| | 1.2 | Specificity-Part-B | 2516 | 1.15 | 0.15 |
| 2 | **Linearity and Range** | | 2592 | 1.02 | 1.5 |
| 3 | **Accuracy study** | | 2562 | 1.20 | 0.69 |
| 4 | **Precision** | | | | |
| | | Method precision (Repeatability) | 2624 | 1.02 | 1.2 |
| | | Intermediate Precision (Ruggedness) | 2685 | 1.01 | 0.74 |
| | | Method equivalency | 2541 | 1.01 | 0.92 |
| 5 | **Solution Stability** | | | | |
| | 5.1 | Initial | 2515 | 1.15 | 0.58 |
| | 5.2 | After 24 hours | 2710 | 1.16 | 0.64 |
| | 5.3 | After 48 hours | 2617 | 1.14 | 0.61 |
| 6 | **Filter study** | | | | |
| | 6.1 | Filtered sample | 2649 | 1.17 | 0.71 |
| | | Centrifuged sample (Unfiltered Sample) | 2596 | 1.36 | 0.75 |
| 7 | **Robustness** | | | | |
| | | Change flow rate by ± 10% (1.4 ml/minute and 1.6 ml/minute). | 2591 | 1.13 | 0.68 |
| | 7.2 | Change the column temperature by ± 5°C (45°C and 35°C) | 2584 | 1.16 | 0.64 |

**Table 42: Data indicating results of validation parameter with acceptance criteria.**

| Characteristics | Acceptance Criteria | Results |
|---|---|---|
| **Specificity** | Peak purity Factor ≥ 995 | **999.99** |
| **Linearity** | Correlation coefficient $r^2 > 0.995$ | 0.9998 |
| **Accuracy/trueness** | Recovery 98-102% (individual and Mean)<br>RSD < 2% | 99.4<br>0.62 |
| **Method precision** | RSD < 2% | 1.20 |
| **Intermediate Precision** | RSD < 2%<br>Overall RSD < 2%<br>Difference of mean assay < 2% | 0.74<br>0.72<br>0.20 |
| **Solution stability** | difference in the response of standard and sample preparation < 2% > 24 h or >12 h | Standard: 0.1<br>Sample: 0.1<br>48 hours |
| **Filter study** | Difference in the mean assay of filtered and unfiltered sample < 2% | 0.5 |
| **Robustness** | Theoretical plates: > 2500<br>% RSD of five replicates : < 2%<br>Asymmetry (tailing factor): < 2% | Meets |

## SUMMARY AND CONCLUSION

There is no interference of blank and placebo peaks with the main peak. All impurities are not detected by this method. The main peak purity is well within the limit of acceptance criteria. The results obtained are well within acceptance criteria. Hence the method cannot be termed as specific.

- A Reverse Phase HPLC Method was developed for simultaneous estimation of [D2122] in tablet formulation. The separation was achieved by Inersil ODS $C_{18}$ Column and Buffer: ACN: IPA (78:12:10 v/v) as mobile phase, at a flow rate of 1.5 ml/min. The detection was carried out at 225nm. The retention time of Levodopa, Carbidopa and Entacopne was found to be at 1.8min, 2.8min and 6.6min.
- **Specificity:** There was no any interference from peaks due to blank, placebo and impurities with the main peak. And peak purity was found to be 1.000.These result indicate specificity of method.
- **Linearity:** was assessed by a plot of concentration versus area. The graphs were found to be linear in range of 40-240µg/ml for Levodopa, 10-60 µg/ml for Carbidopa and 80-480µg/ml for Entacopne with correlation coefficient values 0.99832, 0.9993 and 0.9992 respectively.

## REFERENCES


1. Kamal A, Sagar"levodopa &carbidopa and their metabolites resolution" 2nd edition, 2022; Vol(12): 10-12
2. Kati S, "n-in-one-incubations in determination of intrinsic clearance" 7th edition, 2021; Vol(33): 263-273
3. Sunil P "Development of sensitive, stability-indicating RP-HPLC" 5th edition, 2012; Vol(21).
4. Vaibhav S. Adhao"RP-HPLC method for determination of safinamide mesylate" 21st edition, 2013.
5. S. Marakatham, R.V Vallikumari; Spectrophotometric Method for Determination of Eltrombopag in Bulk and Pharmaceutical Formulation; International Journal of Research in Pharmacy and Biosciences Volume 4, Issue 1, January, 2017; PP 13-16.
6. Rambabu Maddela , Ramakrishna Gajula, Liquid chromatography–tandem mass spectrometric assay for eltrombopag in 50 µL of human plasma: A pharmacokinetic study; Journal of Pharmaceutical and Biomedical Analysis, 2014; 98: 68–73
7. Narottam Pal, P. Pravalika; New Method Development And Validation For The Determination Of Eltrombopag In Bulk And Tablet Dosage Form By Hplc; World Journal Of Pharmacy And Pharmaceutical Sciences; 2018; 7(10).
8. Mastanamma SK, Chandini SK, Reehana SK, Saidulu P. Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of Sofosbuvir and Ledipasvir in bulk and their combined dosage form. Futur J Pharm Sci., 2018; 4(2): 116-123.
9. Shetty R, Jagadish PC. Bioanalytical Method Development and Validation for the Simultaneous Estimation of Zidovudine and Abacavir in Human Plasma by RP-HPLC. Manipal Journal of Pharmaceutical Sciences, 2019; 5(2): 38-46.
10. Nadig S, Jacob JT. A Stability Indicating RP-HPLC Assay Method for Simultaneous Estimation of Abacavir, Lamivudine, Nevirapine and Zidovudine in Pharmaceutical dosage form. Research J. Pharm. and Tech, 2016; 9(11): 1985-1990.