www.ijramr.com

ISSN : 2350-0743

*International Journal of Recent Advances in Multidisciplinary Research*

*Vol. 10, Issue 10, pp.9020-9024, October, 2023*

# RESEARCH ARTICLE

## SIMULTANEOUS ESTIMATION OF LEVODOPA AND BENSERAZIDE BY USING REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY IN API AND MARKETED FORMULATION

**Banothu Bhadru[1*], Venkata Rao, V.[2] and Narender Boggula[1]**

[1]CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad, Telangana, India-501401
[2]Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chandramoulipuram, Chowdavaram, Guntur, Andhra Pradesh, India-522019

**ARTICLE INFO**

***Article History:***
Received 28th July, 2023
Received in revised form
28th August, 2023
Accepted 29th September, 2023
Published online 30th October, 2023

***Key Words:***

Benserazide, Levodopa, RP-HPLC, Validation, Linearity.

**ABSTRACT**

Pharmaceutical analysis plays a vital role in the Quality Assurance and Quality control of bulk drugs. A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of benserazide and levodopa, in its pure form as well as in tablet dosage form. Chromatography was carried out on an Altima $C_{18}$ (4.6 x 150mm, 5µm) column using a mixture of methanol: TEA buffer pH 4.5: acetonitrile (50:25:25) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 225nm. The retention time of the benserazide and levodopa was 2.102, 3.537±0.02min respectively. The method produces linear responses in the concentration range of 5-25mg/ml of benserazide and 12.5-62.5mg/ml of levodopa. The method precision for the determination of assay was below 2.0 %RSD. The method is useful in the quality control of bulk and pharmaceutical formulations. The validated method was successfully applied to quantify levodopa and benserazide simultaneously in a pharmaceutical formulation. The method was found to be precise, sensitive and accurate for the simultaneous determination levodopa and benserazide in bulk and pharmaceutical formulations.

## INTRODUCTION

L-DOPA (L-3,4-dihydroxyphenylalanine) is a chemical that is made and used as part of the normal biology of some animals and plants. Some animals including humans make it via biosynthesis from the amino acid L-tyrosine. L-DOPA is the precursor to the neurotransmitters dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline) collectively known as catecholamines. L-DOPA can be manufactured and in its pure form is sold as a psychoactive drug with the INN levodopa; trade names include Sinemet, Parcopa, Atamet, Stalevo, Madopar, Prolopa, etc.). As a drug it is used in the clinical treatment of Parkinson's disease and dopamine-responsive dystonia. It is soluble in water, DMSO, and 0.5M HCl, insoluble in ethanoland chloroform. Molecular formula is $C_9H_{11}NO_4$,pKa value is 2.32. IUPAC name of levodopa is (2S)-2-amino-3-(3, 4-dihydroxyphenyl) propanoic acid. Mechanism of action is striatal dopamine levels in symptomatic parkinson's disease are decreased by 60 to 80%, striatal dopaminergic neurotransmission may be enhanced by exogenous supplementation of dopamine through administration of dopamine's precursor, levodopa. A small percentage of each levodopa dose crosses the blood-brain barrier and is decarboxylated to dopamine. This newly formed dopamine then is available to stimulate dopaminergic receptors, thus compensating for the depleted supply of endogenous endogenous dopamine[1-5].

Benserazide is used as DOPA decarboxylase inhibitor. It is soluble in water and methanol, insoluble in100% ethanol and acetone.Molecular formula is $C_{10}H_{15}N_3O_5$.IUPAC name is (RS)-2-Amino-3-hydroxy-N′- (2,3,4-trihydroxybenzyl) propanehydrazide. Mechanismof action is it inhibits the aforementioned decarboxylation, and sinceit cannot cross the blood–brain barrier, this allows dopamine to build up solely in the brain instead. Adverse effects caused by peripheral dopamine, such as vasoconstriction, nausea, and arrhythmia, are minimized [6-9].

## MATERIALS AND METHODS

### Chemicals and reagents

**Instrumentation:** HPLC Waters Alliance 2695 separation module, software is Empower 2, 996 PDA detector and Weighing machine isSartorius.

**Preparation of buffer (pH-4.5):** Dissolve 1.5ml of triethyl amine in 250ml HPLC grade water and adjust the pH 4.5.

**Preparation of mobile phase:**Accurately measured 400ml of methanol, 200ml of triethylamine buffer and 400ml of acetonitrile were mixed and degassed in digital Ultrasonicator for 10min and then filtered through 0.45µ filter under vacuum filtration.

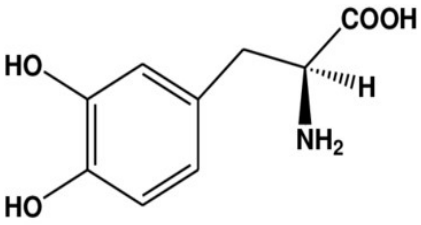


**Figure 1. Molecular structure of levodopa**

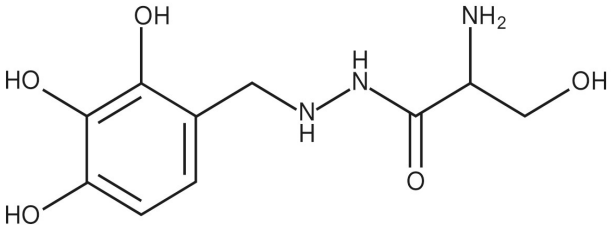


**Figure 2. Chemical structure of benserazide**

**Table 1. Chemicals and solvents used**

| S. No. | Chemicals and solvents | Manufacturer |
|---|---|---|
| 1 | HPLC grade Methanol | Merck |
| 2 | HPLC grade Acetonitrile | Rankem |
| 3 | HPLC grade Water | Merck |
| 4 | Benserazide and levodopa | Gift samples |

**Preparation of standard solution[18-20]:** Accurately weigh and transfer 10mg of benserazide and 10mg of levodopa working standard into a 10ml of clean & dry volumetric flask, add about 7mL of diluents and sonicate to dissolve it completely and make the volume up to the mark with the same solvent(stock solution).Further pipette out 0.1ml of the benserazide and 0.375ml of the levodopa stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

**Optimized chromatographic conditions:** Waters HPLC with auto sampler and PDA Detector, at 40°C, mobile phase ratio is methanol: TEA buffer: acetonitrile (50:25:25 v/v),flow rate 1ml/min, $\lambda_{max}$ set as 225nm, inj. vol. is 10µl and run time is 7min.

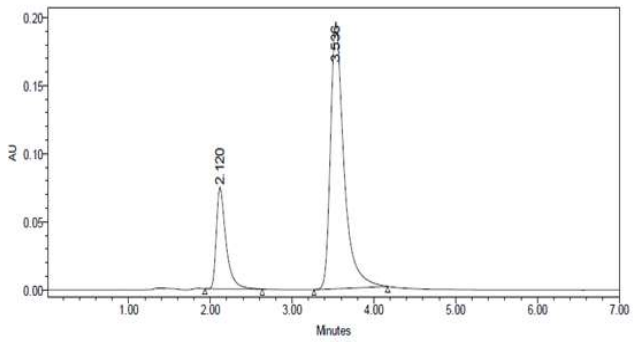


**Figure 3. Chromatograms of benserazide and levodopa**

**Table 2. Optimized method results of benserazide and levodopa**

| S. No. | Peak name | $R_t$ | Area | Height | USP resolution | USP tailing | USP plate count |
|---|---|---|---|---|---|---|---|
| 1 | Benserazide | 2.120 | 775610 | 130275 | 4.0 | 0.98 | 6253 |
| 2 | Levodopa | 3.536 | 555592 | 93740 | 5.06 | 1.23 | 7836 |

# RESULTS

$$\%Assay = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

**Method validation:** The proposed method was validated as per ICH Q2 guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experimental work.

**Specificity:** The specificity studies were carried out to check the interference due to presence of excipients in formulation. The excipients should not show any interference with the drug peak and thus it is called as specificity. Figure 4shows the respective chromatogram for standard, formulation and blank. The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

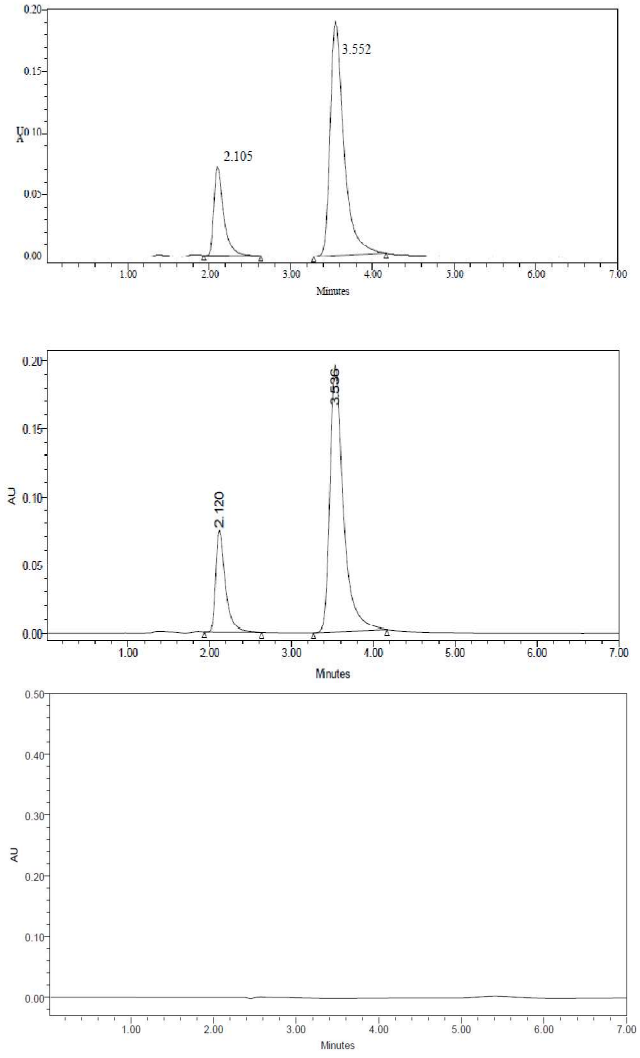


**Figure 4. Chromatogram showing standard, sample and blank**

**Accuracy:** Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions and the % recovery was calculated. Recorded the chromatograms and measured the peak responses. Calculate the amount found and amount added for benserazide and levodopaarecalculating the individual recovery and mean recovery values the percentage recovery

was found to be within the limit (98.6-100.8%), hence this method was accurate.

**Linearity:** The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range of 5-25µg/ml,10-50µg/mlofbenserazide and levodoparespectively. Linearity may be established for all active substances, preservatives and expected impurities. Correlation coefficient ($r^2$) are 0.999&9991, and the intercept are 13756&33265 respectively. These values meet the validation criteria.

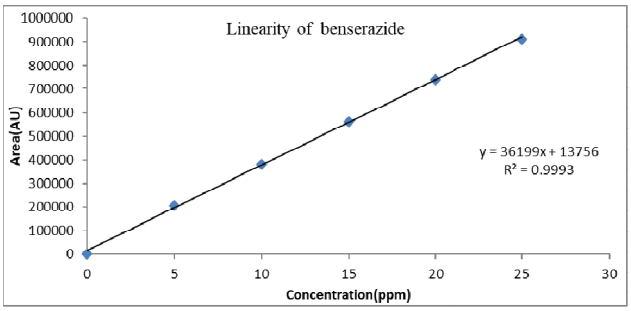


**Figure 5. Calibration graph for benserazide**

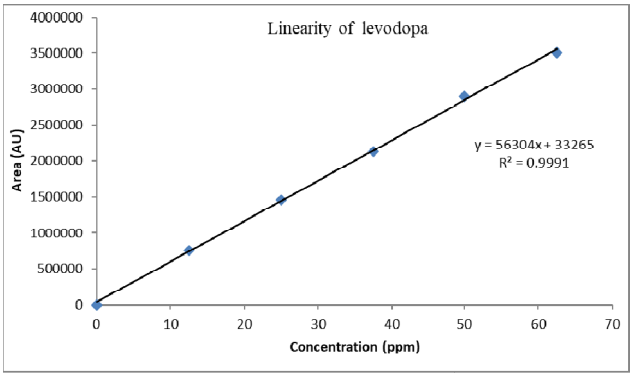


**Figure 6. Calibration graph for levodopa**

**Precision:** The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

**Repeatability:** The method operating over a short tern interval under the same condition. Repeatability is also termed intra-assay precision. It should be obtained by five replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD. Which is within the limits hence method is precise.

**Limit of detection (LOD):** The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

**LOD = 3.3 × σ/s**

Where, σ = Standard deviation of the response, and
S = Slope of the calibration curve.

**Limit of quantification (LOQ):** The quantization limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

**LOQ=10×σ/s**

Where, σ = Standard deviation of the response, and
S = Slope of the calibration curve.

**Robustness:** The robustness was performed for the flow rate variations from 0.9ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Benserazide and Levodopa. The method is robust only in less flow condition and the method is robust even by change in the mobile phase ±5%. The standard and samples of benserazide and levodopa were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

## DISCUSSION

To develop a precise, accurate and suitable RP-HPLC method for the simultaneous estimation of levodopa and benserazide different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. The analytical method was developed by studying different parameters.

**Table 3. Accuracy results ofbenserazide and levodopa**

| Drug Name | % Conc. | Peak area | Amount Added (ppm) | Amount found(ppm) | % Recovery | Mean % recovery |
|---|---|---|---|---|---|---|
| Benserazide | 50 | 287774 | 7.5 | 7.56 | 100.8 | **99.6%** |
| | 100 | 551495 | 15 | 14.8 | 98.6 | |
| | 150 | 825175 | 22.5 | 22.4 | 99.5 | |
| Levodopa | 50 | 1104782 | 18.75 | 18.73 | 100 | **100%** |
| | 100 | 2105321 | 37.5 | 37.4 | 99.9 | |
| | 150 | 3211306 | 56.25 | 56.21 | 100 | |

**Table 4. Linearity results of benserazide and levodopa**

| Level | Benserazide Conc. in µg/ml | Average Peak Area | Levodopa Conc. in µg/ml | Average Peak Area |
|---|---|---|---|---|
| I | 5 | 205035 | 10 | 757881 |
| II | 10 | 381239 | 20 | 757881 |
| III | 15 | 561128 | 30 | 1458941 |
| IV | 20 | 740162 | 40 | 2132457 |
| V | 25 | 909922 | 50 | 2901811 |
| Slop(m) | Y = mx + c | 36199 | Y = mx + c | 56304 |
| Intercept(c) | | 13456 | | 33265 |
| $R^2$ | | 0.999 | | 0.9991 |

**Table 5. Results of repeatability for benserazide**

| S. No. | Name | Rt | Area | Height | USP plate | USP tailing |
|---|---|---|---|---|---|---|
| 1 | Benserazide | 2.108 | 602223 | 128898 | 2586 | 1.6 |
| 2 | Benserazide | 2.105 | 607748 | 129233 | 2947 | 1.4 |
| 3 | Benserazide | 2.113 | 607302 | 127409 | 2468 | 1.6 |
| 4 | Benserazide | 2.109 | 608674 | 127047 | 2146 | 1.9 |
| 5 | Benserazide | 2.109 | 607376 | 129859 | 2307 | 1.7 |
| Mean | | | 606665 | | | |
| Std. Dev. | | | 2542.3 | | | |
| % RSD | | | 0.42 | | | |

**Table 6. Results of method precession for levodopa**

| S. No. | Name | Rt | Area | Height | USP plate | USP tailing |
|---|---|---|---|---|---|---|
| 1 | Levodopa | 3.552 | 2220333 | 2231111 | 1.6 | 2371 |
| 2 | Levodopa | 3.550 | 2221573 | 2674210 | 1.6 | 2841 |
| 3 | Levodopa | 3.564 | 2215483 | 2231261 | 1.5 | 2816 |
| 4 | Levodopa | 3.564 | 2217379 | 2421301 | 1.5 | 2872 |
| 5 | Levodopa | 3.565 | 2211255 | 2324710 | 1.6 | 2845 |
| Mean | | | 2217205 | | 1.6 | 2841 |
| Std. Dev. | | | 4100.8 | | | |
| % RSD | | | 0.18 | | | |

**Table 7. LOD and LOQ of benserazide and levodopa**

| | Benserazide | Levodopa |
|---|---|---|
| Slope | 3188.4 | 39656.07 |
| Standard deviation | 36199 | 56304 |
| LOD (µg/ml) | 0.2 | 2.3 |
| LOQ (µg/ml) | 0.8 | 7.04 |

**Table 8. Robustness of benserazide and levodopa**

| Benserazide | | | | | |
|---|---|---|---|---|---|
| **Parameters** | **Changes** | **Peak area** | **Rt** | **SD** | **%RSD** |
| Flow rate as per method 1.0 mL/min | 1.0 mL/min | 607323 | 2.102 | 36198 | 0.40 |
| | 0.9 mL/min | 674735 | 2.330 | 35175 | 0.42 |
| | 1.1 mL/min | 1408920 | 1.950 | 38199 | 0.45 |
| Mobile phase composition | | | | 35175 | 0.42 |
| | Less organic phase | 674735 | 2.290 | 36198 | 0.40 |
| | More organic phase | 1408920 | 1.998 | 38199 | 0.45 |
| **Levodopa** | | | | | |
| Flow rate as per method | 1.0 mL/min | 558777 | 3.537 | 56503 | 0.18 |
| | 0.9 mL/min | 2505636 | 3.885 | 55702 | 0.17 |
| | 1.1 mL/min | 1408920 | 3.263 | 56904 | 0.18 |
| Mobile phase composition | As it is | | | 56703 | 0.17 |
| | Less organic phase | 2239255 | 4.435 | 55705 | 0.18 |
| | More organic phase | 2300346 | 3.009 | 56602 | 0.19 |

First of all, maximum absorbance was found to be at 225nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Altima $C_{18}$ because it was giving good peak.Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is methanol: TEA Buffer pH 4.5: acetonitrile (50:25:25) was fixed due to good symmetrical peak. So, this mobile phase was used for the proposed study. Run time was selected to be 7min because analyze gave peak around 2.102, 3.537±0.02min respectively and also to reduce the total run time.

## CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of benserazide and levodopain bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps. Benserazide and levodopawas freely soluble in ethanol, methanol and sparingly soluble in water. Methanol:TEA Buffer pH 4.5: acetonitrile (50:25:25) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed intablesfor RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the spectrophotometric methods. This method can be used for the routine determination of benserazide and levodopain bulk drug and in pharmaceutical dosage forms.

**Competing interest statement:** All authors declare that there is no conflict of interests regarding publication of this paper.

**Additional information:** No additional information is available for this paper.

**Financial support and Sponsorship:** None.

## REFERENCES


Valko K, Snyder LR, Glajch J. Retention in Reversed-Phase Liquid Chromatography as a Function of Mobile Phase Composition. J. Chromatogr. A. 1993; 656(2):501-520.

Gouthami Thumma, Narender Boggula, Vasudha B, Anand Kumar Chettupalli. Method Development and Validation for the Simultaneous Analysis of Duloxetine HCL and Methylcobalamine by RP-HPLC. International Journal of Pharmaceutical Quality Assurance 2018; 9(4); 363-367.

Kazakevich Y, Lobrutto R. HPLC for Pharmaceutical Scientists. 1stEd. New Jersey: John Wiley & Sons Inc; 2007: p. 987-1051.

Thaidala Sriveni, Vanamala Naveen, Vemula Sai Rupa, Aeruva Renuka, Sunil Porika, M Akiful Haque, Vasudha Bakshi, Narender Boggula. Development and Validation of Dolutegravir in Bulk and Formulation: An Anti-Retroviral Drug Using UV-Spectroscopy. International Journal of Pharmaceutical Quality Assurance. 2021; 12(1):57-60.

Huber JFK, Vander LR, Ecker E, et al. Column switching in High Pressure Liquid Chromatography. J. Chromatogr. A. 1973; 83(2):267-271.

Snyder LR, Schunk TC. Retention mechanism and the role of the mobile phase in normal-phase separation on amino-bonded-phase columns. J. Anal. Chem. 1982; 54(11):1764–1772.

Yun KS, Zhu C, Parcher JF. Theoretical relationships between the void volume, mobile phase volume, retention volume, adsorption and Gibbs free energy in chromatographic processes. J. Anal. Chem. 1995; 67(4):613–619.

Braithwaite A, Smith FJ.Chromatographic Methods. 5thEd.Kluwer Academic Publisher; London: 1996: p. 27-29.

Heinisch S, Rocca JL. Effect of mobile phase composition, pH and buffer type on the retention of ionisable compounds in reversed-phase liquid chromatography: application to method development. J. Chromatogr. A. 2004;1048(2):183-193.

Gritti F, Guiochon G. Role of the buffer in retention and adsorption mechanism of ionic species in reversed phase liquid chromatography. J.Chromatogr A.2004; 1038(1-2):53–66.

BoschE, EspinosaS,RosesM. Retentionof ionizable compoundson high performance liquid chromatography: III. Variation of pKa values of acids and pH values of buffers in acetonitrile-water mobile phases. J. Chromatogr. A. 1998; 824(2):137–146.

Bosch E, Bou P, Allemann H, et al. Retention of ionisable compounds on HPLC: pH scale in methanol–water and the pKa and pH values of buffers. J. Anal. Chem. 1996; 68(20): 3651–3657.

Kupiec T. Quality controlanalyticalmethods:high-performanceliquid chromatography. Int. J. Pharma. Compound. 2004; 8(3):223-227.

Dethy S, Laute MA, Van Blercom N, Damhaut P, Goldman S, Hildebrand J. Microdialysis-HPLC for plasma levodopa and metabolites monitoring in parkinsonian patients. Clin Chem. 1997; 43(5):740-744.

Kumar SS, Natraj K, Khan A, Kumar BK, Rao JV. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Levodopa and Carbidopa in Pharmaceutical Dosage Forms. J. Pharm. Res. 2011; 4(11):1-4.

Prafullachandra T, Vinayak SM, Pai NR., Chandrabhanu M, Smruthi T, Estimation of Entacapone tablets by Reverse Phase High Performance Liquid Chromatography Method. Bioscience Discovery.2011; 2(3):294-298.

Raj Kumar D, Ravi R, Subbu Raju T, Revathi H, Arul C, Gopalakrishnan K. Development and validation of stability indicating RP-HPLC assay method for Levodopa and Carbidopa in Levodopa, Carbidopa and Entacapone ER tablets. Int J of Pharm Res Dev.2013; 4(11):98-105.

Shailesh KK,Ashwin SA, Nurudin PJ, Ravi AM, Shivan, Pandey. Development and validation of derivative spectrophotometric method for determination of entacapone in pharmaceutical formulation. IntJ Pharm Res Dev.2009; 8(1):1-8.

Gowrisankar D, PrakashSS, Saurabh K, Raju SA. Optimization and validation of quantitative spectrophotometric methods for determination of Entacapone. J Ind Council Chem.2009; 26(1):74-76.

Damiani Patricia C, Moschetti Andrea C, Rovetto Adrian J, Benevento F, Olivieri Alejandro C. Design and optimization of a chemometric assisted spectrophotometric method for simultaneous determination of levodopa and carbidopa in pharmaceutical products. Analytical Chemica Acta. 2005; 543(1):192-198.

Kshirsagar VB, Deokate UA, Bharkad VB, Khadabadi SS. HPTLC Method Development and validation for the simultaneous estimation of diosgenin and levodopa in marketed formulation, Asian J Res Chem. 2008; 1(1):36-39.

Naveen Pathakala, Mohammed Sayeed, Ram Mohan Manda, Himabindu Peddapalli, Rajendra Kumar Jadi, Vasudha Bakshi, Narender Boggula. Method Development and Validation for The Determination of Dolutegravir in Bulk Material and in Tablet Dosage Form. Eur. Chem. Bull. 2022; 11(9):82-87.

Srinidhi, M. MD. Mushabbar Basha, V. Raj Kumar, J. Rajendra Kumar.Stability Indicating RP-HPLC Method Development and Validation for the Estimation of Sumatriptan in Bulk and Pharmaceutical Dosage Form. Journal of Applied Pharmaceutical Science. 2016; 6(6):020-025.


*******