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Development and Validation of UV-Visible Spectrophotometric Method for Estimation of Levodopa

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A B S T R A C T

Aim: The aim of the proposed study was to development and validation of simple, accurate, precise yet economical UV-Visible spectrophotometric method for the quantitative estimation of Levodopa.

Methods: A UV–Visible spectrophotometric method was developed using a co-solvent system consisting of 20% methanol and 80% 0.01M potassium hydrogen phosphate buffer (K_2HPO_4). Levodopa solution was scanned over the entire UV-visible range to determine its wave length of maximum absorbance. The method was validated in accordance with the ICH Q2(R1) guidelines which involves various parameters viz. linearity, accuracy, precision, robustness, ruggedness, limit of detection (LOD), and limit of quantitation (LOQ).

Results: The wavelength of maximum absorbance (λmax) for Levodopa was observed at 283 nm using a co-solvent system composed of 20% methanol and 80% 0.01M potassium phosphate buffer (K_2HPO_4).The developed UV–Visible spectrophotometric method demonstrated excellent linearity across the concentration range of 5 to 60µg/mL, with a correlation coefficient (r^2) of 0.999. The intra-day accuracy in terms of % Differencewas in the range of −7.88% to +4.46% and Inter day accuracy was in the range of −7.60% to +2.42%. The precision values(% RSD) were found to be below 3% for both intra- and inter-day studies. Robustness and ruggedness studies confirmed the method's reliability under deliberate variations in analytical conditions with %RSD values below 3%. The limit of detection (LOD) and limit of quantitation (LOQ) were determined to be 0.9189µg/mL and 2.7884µg/mL, respectively, ensuring adequate sensitivity for routine quality control analysis.

Conclusion: A simple, accurate, precise and cost-effective UV-visible spectrophotometric method was successfully developed for the estimation of Levodopa. The method was developed using an economical percentage of the organic phase in co-solvent system. The validated UV-Visible method was found to be efficient for the estimation of Levodopa in the commercial samples. Considering the advantages, said developed method can be used for the routine analysis of Levodopa.

Keywords: Levodopa, UV spectrophotometry, method development, analytical method validation,ICH guidelines.

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INTRODUCTION

Parkinson's disease (PD) is a neurological movement disorder that interferes with the performance of everyday tasks. It arises due to the degeneration of neurons in a particular area of the brain known as the substantia nigra. These neurons are responsible for producing dopamine, a key neurotransmitter that facilitates communication between nerve cells and plays a crucial role in controlling body movement[1]. Currently, several drugs, including Levodopa & carbidopa are used for the treatment of Parkinson's disease. Levodopa, chemically known as (2S)-2-amino-3-(3,4-dihydroxyphenyl) propanoic acid acts as a precursor of dopamine, norepinephrine, and epinephrine, helping to replenish dopamine levels in the brain[2].

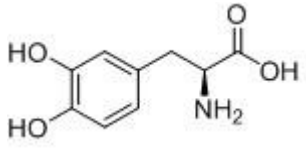

Figure 1: Chemical Structure of Levodopa

There are numerous analytical methods available for the estimation of levodopa but the simple yet precise UV-Visible spectrophotometric methods for the same are very limited. Moreover, the available UV-Visible spectrophotometric methods have slightly higher levels of detection and quantification limits. Some of the methods involves use of complex solvent systems.

In analytical development, the use of eco-friendly and low-cost solvents in UV spectrophotometric methods aligns with green chemistry principles and regulatory standards, offering practical benefits during method validation and routine quality testing in both academic and industrial settings [7-13].

Considering the vitality of Levodopa formulations in the treatment of Parkinsonism, its necessary quality and the analytical method responsible for estimating such quality, it was envisaged that development of simple yet precise and sensitive UV-Visible spectrophotometric methods would be worth.

Material and method

Instrumentation:

A double beam UV-Visible Spectrophotometer of Jasco Inc. (V-530) along with Quartz cells of 3 cm length and 1 cm path length and Spectra Manager software was used for the method development. For accurate weighing, analytical balance of Essae, (Vibra HT) having internal calibration facility was used.

Material: Levodopa was purchased from Divis Laboratories (India) Pvt. Ltd., Hyderabad. HPLC grade methanol was purchased from Avantor, India. AR grade di-potassium hydrogen phosphate (K_2HPO_4) was purchased from Hi Media, India. HPLC grade water was obtained from in-house water purification system (Lablink).

Preparation of standard stock solution:

Initially, 1.36gm $K_2HPO_4$was dissolved in 1000ml HPLC grade water so as to achieve the buffer solution strength of 0.01M. A co-solvent system was prepared by admixing the freshly prepared buffer solution and methanol in a ratio of 80:20 v/v.

100mg of accurately weighed Levodopa was dissolved in100ml co-solvent system so as to achieve the solution of 1mg/ml (Stock-I). Stock-I was diluted suitably with co-solvent system so as to achieve the Levodopa solution of 100µg/ml strength (Stock-II).

Determination of wavelength of maximum absorbance (λ_{max}):

Stock-II solution was diluted suitably with co-solvent system and Levodopa solution of 20µg/ml was achieved. UV-Visible Spectrophotometer was subjected to auto-zero mode using blank co-solvent solution. After auto-zero, Levodopa solution of 20µg/ml was scanned over the UV-Visible range of 800 to 200 nm using Spectrum measurement mode of the UV-Visible Spectrophotometer. While scanning, the medium scanning speed was used for the better results. After acquisition of the UV-Visible spectrum of Levodopa, λ_{max} was identified using the spectral tool of the software.

Preparation of calibration curve:

Stock-II solution was suitably diluted with freshly prepared co-solvent system so as to achieve seven different calibration standard solutions viz.CAL-STD-1 (5µg/mL), CAL-STD-2 (10µg/mL), CAL-STD-3 (20µg/mL), CAL-STD-4 (30µg/mL), CAL-STD-5 (40µg/mL), CAL-STD-6 (50µg/mL) and CAL-STD-7 (60µg/mL). The absorbance of each calibration standard was recorded at previously determined $\lambda_{max}$of 283 nm. To evaluate the reproducibility of the method, the entire procedure was repeated five times under the above-mentioned identical conditions.

Method Validation

The developed UV spectrophotometric method for Levodopa estimation was validated in accordance with ICH Q2(R1) guidelines. Key validation parameters including linearity, accuracy, precision, robustness, ruggedness, limit of detection (LOD), and limit of quantitation (LOQ) were systematically evaluated.

Linearity and Range:

Linearity of the proposed UV spectrophotometric method was evaluated using above mentioned seven calibration standards across a defined concentration range. A calibration curve was constructed by plotting absorbance against concentration, and linearity was assessed through linear least-squares regression analysis. The coefficient of determination (R^2) was used as a measure of the method's linearity. The analytical range was defined as the interval between the lowest and highest concentrations within which the method demonstrated acceptable linearity and response.

Accuracy:

Accuracy of the method was established using three different quality control standards (QC-STD). Stock-II solution of Levodopa was suitably diluted so as to achieve QC-STD-1 (7µg/ml), QC-STD-2 (28µg/ml) and QC-Std-3 (58µg/ml). For the intra-day studies, all the QC-STDs (n = 5) were analyzed using developed UV method for its content at three different time intervals (morning, afternoon, and evening)in a day. For the inter-day studies, the process of analyzing QC-STDs was repeated on the three consecutive days.

Intra-day and inter-day accuracy of the method was determined by comparing the nominal concentrations of Levodopa quality control samples with their corresponding mean measured values. Accuracy was expressed as percent difference (% diff) to assess the closeness of the measured values to the true concentrations.The % difference was calculated using the following formula

$$\%\ Difference = \frac{(Mean\ Measured\ Concentration - Nominal\ Concentration)}{Nominal\ Concentration} \times 100 \ldots \ldots\ldots (1)$$

Precision: The intra-day and inter-day precision of the proposed UV spectrophotometric method was established by analyzing the above mentioned three QC-STDs (n = 5) for its content at three different time intervals (morning, afternoon, and evening) on the same day and for the next two consecutive days. The precision was expressed in terms of percent relative standard deviation (%RSD).

The % RSD was calculated using the following formula.

$$\% RSD = \frac{Standard\ Deviation(SD)}{Mean} \times 100 \ldots\ldots\ldots (2)$$

The method was considered to be precise in case of %RSD values ≤ 5 for the contents of all the three QC-STDs.

Robustness:

Robustness of the developed UV spectrophotometric method was demonstrated by introducing minor, deliberate changein the methanol percentage in the co-solvent system. The methanol content in the co-solvent system was adjusted to either 18% or 22% and two different co-solvent systems having different percentage of methanol were prepared. The three QC-STDs (n = 5) as mentioned earlier were prepared using two different co-solvent systems as mentioned above. All the QC-STDs were analyzed for its contents and the results were expressed in terms of mean ± % RSD. The method was considered to be robust in case of %RSD values ≤ 5 for the contents of all the three QC-STDs.

Ruggedness:

Additionally, aruggedness of the proposed UV method was established by analyzing Levodopa solution (28µg/mL) in triplicates by using two distinct instruments (BIOAGE and V-530 JASCO) and two in-house analysts. The results were expressed in terms of mean ± % RSD.The method was considered to be rugged in case of %RSD values ≤ 5.

Limit of Detection (LOD): The LOD of the developed UV method was calculated by using the following formula,

$$LOD = \frac{3.3 \times SD}{S} \ldots\ldots\ldots (3)$$

Where, SD= Standard deviation of Y-intercepts

S= Slope of the calibration curve

Limit of Quantitation (LOQ): The LOQ of the developed UV method was calculated by using the following formula

$$LOQ = \frac{10 \times SD}{S} \ldots\ldots\ldots (4)$$

Where, SD= Standard deviation of Y-intercepts

S= Slope of the calibration curve

Estimation of Levodopa in marketed formulations:

Proposed validated UV-Visible spectrophotometric method was used for the estimation of Levodopa in its commercial, marketed formulations. Tablet formulations of three different brands of the Levodopa having 100 and 200 mg strength were procured from the local market of the Chhatrapati Sambhajinagar, Maharashtra, India. Five tablets of each brand were selected for the studies. The contents of each tablet were collected and weighed separately. The contents equivalent to 50 mg of the Levodopa were dissolved in co-solvent system containing buffer and methanol (80:20 v/v) and diluted to 50 ml using calibrated volumetric flask. The solutions were subjected to ultra-sonication for 15 minutes using ultrasonic water bath. After sonication, solutions were filtered through 0.22 µm syringe filter. Resultant solutions were suitably diluted using co-solvent system. Resulted solutions were analyzed for its Levodopa content using pre-validated UV-Visible spectrophotometric method.

RESULTS AND DISCUSSION

Determination of the wavelength of maximum absorbance:

Accurate quantitative analysis by the UV-Visible spectrophotometry requires the selection of an appropriate wavelength where the analyte exhibits maximum absorbance. In the proposed study, Levodopa in the presence of co-solvent system consisting of K_2HPO_4 buffer solution and the methanol (80:20 v/v) when scanned for the UV-Visible range of 800 to 200nm, showed λ_{max} of 283nm. In order to assess the reliability of the results, experiments were repeated five times which upon assessment confirmed that 283nm is the $\lambda_{max}$of Levodopa under the above mentioned specified conditions.

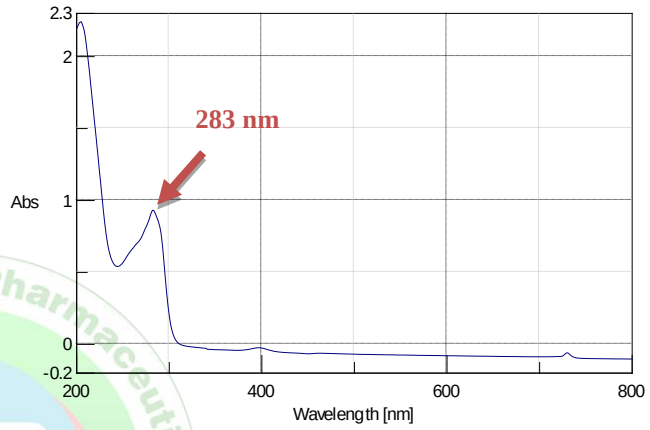

Figure 2: Representative Full Scan UV-Visible Spectrum of Levodopa

Preparation of a calibration curve

To facilitate the quantification of unknown concentrations of Levodopa by theproposed UV-method, a seven-point calibration curvewas established. The mean ± S.D. values against each calibration standard are depicted in the Table No. 1.

Table 1: Calibration standard data for Levodopa

Calibration Standard (No.)	Concentration (µg/mL)	Absorbance (Mean ± S.D.)
CAL-STD-1	5	0.0802± 0.0083
CAL-STD-2	10	0.1790 ± 0.0103
CAL-STD-3	20	0.3555 ± 0.0073
CAL-STD-4	30	0.5537 ± 0.0073
CAL-STD-5	40	0.7126 ± 0.0064
CAL-STD-6	50	0.8962 ± 0.0059
CAL-STD-7	60	1.0511 ± 0.0155

Method validation:

In order to check the reliability and the authenticity of the proposed UV-Visible Spectrophotometric method of Levodopa, it was validated using ICH Q2(R1) guidelines. The proposed method was assessed using variety of parameters.

Linearity and Range:

The linearity and the range of the proposed method was established over the seven-point calibration curve which covers the Levodopa concentrations in the range of 5 to 60µg/mL. The five calibration curves showed excellent

linearity over the entire range of calibration standards ($r^2 > 0.999$) with consistent slopes and intercepts, confirming the method's linearity and range as illustrated in figure 3 (A-E).

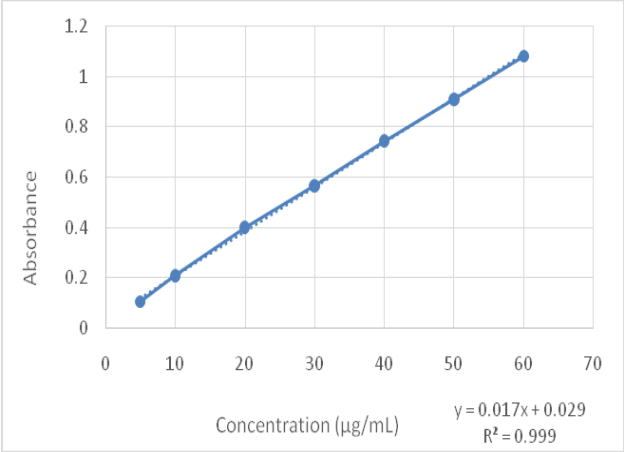

Figure 3: (A) Calibration curve replicate 1

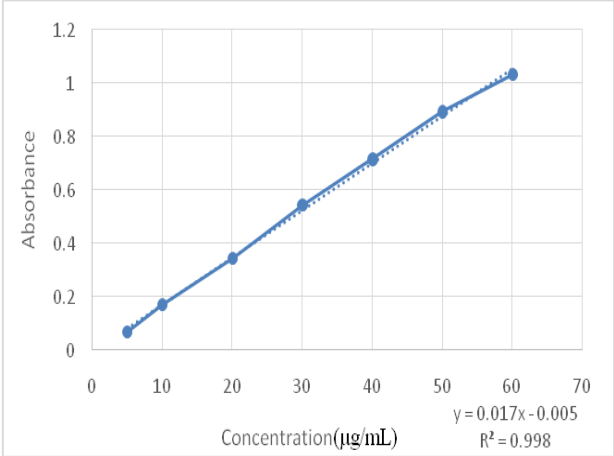

Figure 3: (C) Calibration curve replicate 3

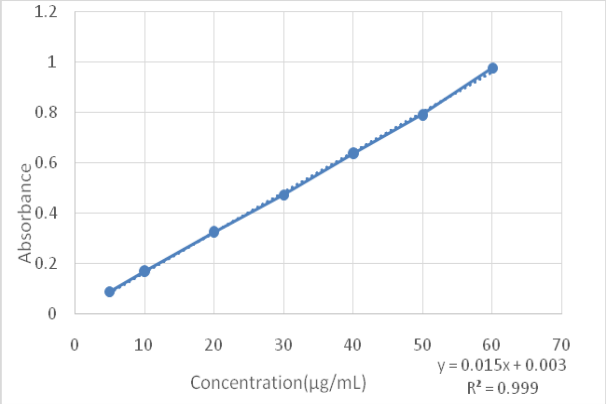

Figure 3: (B) Calibration curve replicate 2

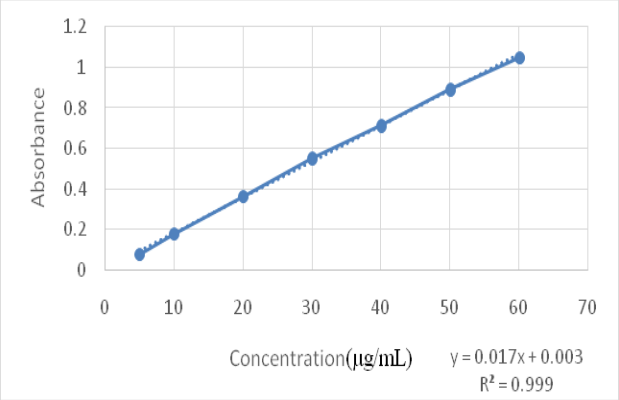

Figure 3: (D) Calibration curve replicate 4

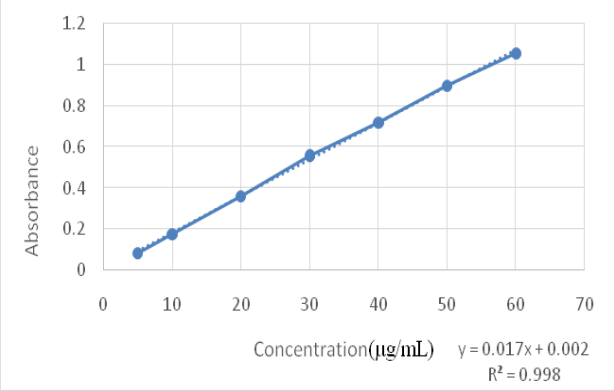

Figure 3: (E) Calibration curve replicate 5

According to linearity analysis, the proposed UV method of Levodopa was found to be linear over the pre-defined concentration range of calibration standards.

Accuracy:

Accuracy is defined as the closeness of agreement between the experimentally obtained values and the true or nominal concentrations. Ensuring accuracy across the entire calibration range is essential for the reliability of the analytical method at all stages of quantification. In the present study, intra-day accuracy values (% difference) were found to be in the range of −7.88% to +4.46%, while inter-day accuracy values were found to be in the range of −7.60% to +2.42. Taking into the consideration of narrow range of % difference values, the proposed UV spectrophotometric method for Levodopa was claimed to be accurate and reliable for its routine use in pharmaceutical analysis.

Table 2: Intra- day accuracy data of the UV method for Levodopa

Level of QC Standard	Nominal Concentration (µg/mL)	Mean Measured Concentration (µg/mL)	% Difference
LQC	7	6.85	-2.09
	7	6.45	-7.88
	7	6.50	-7.12
MQC	28	28.86	3.08
	28	29.25	4.46
	28	27.18	-2.91
HQC	58	57.06	-1.62
	58	57.68	-0.55
	58	56.14	-3.21

Table 3: Inter- day accuracy data of the UV method for Levodopa

Level of QC Standard	Nominal Concentration (µg/mL)	Mean Measured Concentration (µg/mL)	% Difference
LQC	7	6.47	-7.60
	7	6.64	-5.20
	7	6.70	-4.30
MQC	28	28.49	1.76
	28	28.68	2.42
	28	28.31	1.10
HQC	58	56.50	-2.59
	58	57.02	-1.68
	58	57.36	-1.11

Precision: Precision reflects the degree of variability among repeated measurements, indicating the consistency and reliability of an analytical method. A validated method is expected to yield reproducible results under the same experimental conditions. In the present study, the intra-day and inter-day precision of the developed UV spectrophotometric method was assessed using Levodopa QC-STDs. Intra-day precision values of proposed UV Visible spectrophotometric method were found to be in the range of 0.18 to 2.89,whereas inter-day precision of the proposed method was found to be in the range of 1.20 to 2.97. The detailed results of intra-day and inter-day precision studies of proposed UV Visible spectrophotometric methods are summarized in Table No. 4 and 5 respectively.

Table 4: Intra-day precision data of the UV method for Levodopa

	Day 1			Day 2			Day 3		
QC-STD (µg/mL)	Mean	SD	% RSD	Mean	SD	% RSD	Mean	SD	% RSD
7	6.85	0.20	2.89	6.45	0.14	2.23	6.50	0.08	1.18
28	28.86	0.17	0.58	27.18	0.23	0.79	27.18	0.42	1.55
58	57.06	0.79	1.39	56.14	0.47	0.82	56.14	0.10	0.18

Table 5: Inter-day precision data of the UV method for Levodopa

	Morning			Afternoon			Evening		
QC-STD(µg/mL)	Mean	SD	% RSD	Mean	SD	% RSD	Mean	SD	% RSD
7	6.47	0.16	2.41	6.64	0.16	2.45	6.70	0.19	2.84
28	28.49	0.74	2.59	28.68	0.84	2.93	28.31	0.84	2.97
58	56.50	0.68	1.20	57.02	0.70	1.22	57.36	0.92	1.60

Robustness:

Robustness refers to the ability of an analytical method to remain unaffected by small, deliberate variations in experimental conditions, there by demonstrating its reliability during routine usage. Minor changes in parameters such as pH or solvent composition are common in practical scenarios and should not compromise the method's overall performance. In the proposed study, the robustness of the UV spectrophotometric method was evaluated by altering the composition of the co-solvent system. Specifically, the methanol content was varied slightly from 18% to 22% (v/v) to simulate potential fluctuations. As shown in Table 6, the % RSD values for absorbance measurements were found to be in the range of 0.30 to 3.92 which are within the acceptable limit of ≤5. Based on the obtained results, it was concluded that proposed method is robust.

Table 6: Robustness data of the UV method for Levodopa.

QC-STD(µg/mL)	Co-solvent Composition (Methanol: 0.01M K_2HPO_4 v/v)	Absorbance (Mean ± S.D.)	% RSD
7	18:82	0.1268 ± 0.0016	1.26
28	18:82	0.5052± 0.0021	0.41
58	18:82	1.0047± 0.0039	0.38
7	22:78	0.1247± 0.0049	3.92
28	22:78	0.4945± 0.0039	0.78
58	22:78	1.0009± 0.0031	0.30

Ruggedness:

An analytical method's ruggedness is its capacity to withstand changes in external factors that affect its performance, such as modifications to labs, equipment, and analysts.The robustness of the suggested UV-visible method was assessed by analyzing Levodopa solution (28µg/mL) (n = 3) using two distinct UV-visible spectrophotometers and recording the results in terms of mean absorbance ± SD and the %RSD (Table No. 7). The %RSD values as depicted in Table No. 7 are within the acceptable limit of ≤5. Based on the results, it was envisaged that the proposed UV-visible spectrophotometric method is rugged in nature.

Table 7: Ruggedness data of the UV method for Levodopa.

Levodopa Concentration (µg/mL)	Model &Make of the UV-Visible Spectrophotometer Used	Absorbance (Mean ± S.D.)	% RSD
28	V-530, Jasco Inc.	0.4987 ± 0.0029	0.5815
28	UV 2600, BioAge	0.4912 ± 0.0031	0.6311

Limit of Quantification (LOQ) and Limit of Detection (LOD): The lowest concentration that can be examined with reasonable accuracy and precision is represented by LOQ. Table No. 8 depicts the LOD and LOQ of the proposed UV Visible spectrophotometric method, which were determined to be 0.9289µg/mL and 2.7884µg/mL, respectively.

Table 8: LOD & LOQ data for UV method for Levodopa.

Parameter	Values Obtained
LOD	0.9289µg/mL
LOQ	2.7884µg/mL

The lower limits of proposed UV Visible spectrophotometric method for detection and quantification of Levodopa suggest that the said method would be appropriate for assessing materials containing lower amounts of Levodopa.

Estimation of Levodopa in marketed formulations:

Newer analytical methods are generally developed and validated for its potential application in routine analysis. Before its regular application, it is necessary to establish its reliability to analyze the routine samples. Accordingly, the proposed analytical method of Levodopa was utilized for the analysis of marketed formulations of the Levodopa. The results of the analysis of marketed formulations are depicted in Table 9. The proposed method was able to estimate the Levodopa content in the three marketed formulations with sufficient accuracy as the obtained results are matching with the label claims of the commercial formulations.

Table 9: Levodopa content in commercial formulations

Sr.	Formulation	Brand Name	Label Claim (Levodopa Content)	% Assay (Mean ± S.D.)
1.	Tablet	Dopar	200 mg	98.29 ± 0.505
2.	Tablet	Syndopa	100 mg	96.87 ± 1.511
3.	Tablet	Levopa	200 mg	98.23 ± 1.651

CONCLUSION

The proposed UV-visible spectrophotometric method for the estimation of Levodopa is simple, sensitive, accurateand precise. It is robust and rugged which confirms its suitability for the routine quantitative analysis of Levodopa in pharmaceutical formulations.

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CONFLICT OF INTEREST

Regarding the research authorship and/or publication of this paper, the author(s) have stated that they have no potential conflicts of interest.

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