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Research Article

UV-Visible Spectrophotometric Method Development and Validation for The Estimation of Levodopa in Nasal Medium

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Abstract

Objective: The objective of this study was to develop a straightforward UV-Visible spectrophotometric method for the quantitative analysis of Levodopa in both its pure form and pharmaceutical dosage forms, followed by validation of the proposed analytical method in accordance with standard guidelines.

Material and Methods: Quantitative analysis of Levodopa was performed using a UV-Visible double-beam spectrophotometer, with measurements taken at the maximum absorbance wavelength (280 nm) in phosphate-buffered saline (pH 6.4) to simulate the nasal environment. The drug was initially characterized through melting point determination using the capillary fusion method and further confirmed by Fourier Transform Infrared (FTIR) spectroscopy. A novel UV-Visible spectrophotometric method was developed for the estimation of Levodopa, and its validation was conducted in accordance with International Conference on Harmonisation (ICH) guidelines, evaluating key analytical parameters such as linearity, accuracy, precision, repeatability, sensitivity, robustness and ruggedness.

Result: The melting point of the drug was determined to be 277 °C, which aligns with its reported reference range, confirming its identity. Further structural confirmation was achieved through spectral interpretation. A linear relationship was observed for Levodopa in the concentration range of 1–6 µg/mL, exhibiting a regression coefficient (R^2) of 0.9988, indicating strong linearity. The precision of the method was assessed, with intraday and interday relative standard deviation (RSD) values found to be 0.734% and 0.720%, respectively—both within acceptable limits. To evaluate the sensitivity of the developed method, the limit of detection (LOD) and limit of quantification (LOQ) were calculated as 0.810 µg/mL and 2.452 µg/mL, respectively.

Conclusion: The UV spectrophotometric method developed and validated for the analysis of Levodopa demonstrated acceptable linearity, accuracy, and precision, and proved to be cost-effective, making it suitable for routine quality control and quantitative evaluation of its pharmaceutical formulations.

Keywords: UV visible spectrometric, Levodopa, validation, nasal media, saline phosphate buffer pH 6.4

INTRODUCTION

Levodopa is a therapeutic agent classified under medications that influence the central nervous system. It is the L-isomer of dopa, exhibiting optical activity due to its specific spatial configuration¹. This compound is primarily employed in managing symptoms associated with Parkinson's disease, including muscle rigidity,

involuntary movements, tremors, and impaired coordination². Levodopa functions in various biological roles, such as serving as a prodrug, hapten, neurotoxic compound, antiparkinsonian agent, dopaminergic modulator, antidyskinesia treatment, allelochemical, plant growth inhibitor, and as a metabolic product in humans, mice, and plants³.

Levodopa is classified as a dopa compound, specifically a derivative of L-tyrosine and a non-proteinogenic L-alpha-amino acid. It exists as the conjugate acid form of L-dopa and is the mirror image (enantiomer) of D-dopa. Additionally, it has a tautomeric relationship with the zwitterionic form of L-dopa. The IUPAC designation for Levodopa is (S)-2-Amino-3-(3,4-dihydroxyphenyl) propionic acid, it is also commonly referred to as L-3,4-dihydroxyphenylalanine, and structure of levodopa is displayed in figure 1. Its chemical formula is $C_9H_{11}NO_4$, and it has a molecular weight of 197.19 g/mol.⁴

Chemical properties: Levodopa crystallizes from aqueous solutions as colourless, odourless, and tasteless needle-like structures. It exhibits a boiling point of approximately 448.4°C (838.4°F) and undergoes decomposition at a melting point range of 276–278°C (528.8–532.4°F)⁵. The compound demonstrates high solubility in dilute mineral acids such as hydrochloric and formic acid, whereas it is practically insoluble in organic solvents including ethanol, benzene, chloroform, and ethyl acetate. Its aqueous solubility is reported as 66 mg per 40 mL. In the presence of atmospheric moisture, L-dopa is prone to rapid oxidative degradation by molecular oxygen, resulting in discoloration due to the formation of oxidation products.

Mechanism of action of levodopa: Levodopa (L-dopa) functions as the immediate biosynthetic precursor to dopamine and constitutes the primary therapeutic agent in the management of Parkinson's disease—a progressive neurodegenerative disorder characterized by the selective and gradual loss of dopaminergic neurons in the pars compacta of the substantia nigra, leading to a marked depletion of striatal dopamine levels, resulting in reduced dopamine levels⁶.

Following oral administration, levodopa is absorbed in the small intestine and transported across the blood-brain barrier (BBB) by an active carrier system specific for large neutral amino acids. Within the central nervous system (CNS), levodopa undergoes enzymatic Carboxyl group removal by aromatic L-amino acid decarboxylase (AADC) to form dopamine, which then exerts its therapeutic effects by replenishing depleted dopamine stores in the brain⁷.

As dopamine itself is unable to traverse the BBB, levodopa acts as a prodrug, enabling central dopaminergic activity. The restored dopamine levels aid in improving motor symptoms such as tremors, muscle rigidity, and bradykinesia that are characteristic of Parkinsonian syndromes.

To enhance CNS bioavailability and minimize peripheral side effects due to systemic conversion of levodopa to dopamine, Levodopa is typically co-administered with peripheral aromatic L-amino acid decarboxylase (AADC) inhibitors, such as carbidopa or benserazide, which limit its extracerebral decarboxylation. These inhibitors do not cross the blood-brain barrier, thereby preventing peripheral conversion to dopamine while preserving central nervous system availability, thereby increasing the proportion of the drug that reaches the brain in its active form⁸.

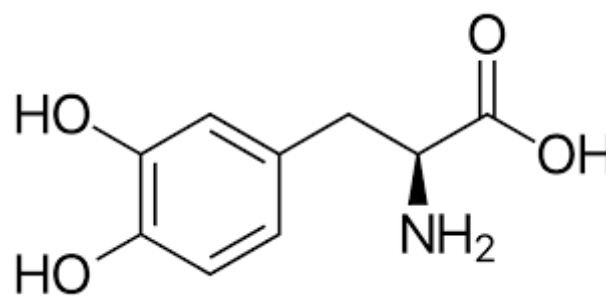


Figure 1: Structure of levodopa

MATERIALS AND METHODS

Instruments and reagents

A double beam UV-visible spectrometer (Shimadzu, Japan, UV-1800 240V) with spectra manager software were used for the analysis. Quartz cells having 3cm length with 1cm path length were used for spectral measurement⁹. Weighing balance (WENSAR) with internal calibration mode was used for the accurate weighing purpose. Levodopa was obtained as gift sample from Intas Pharmaceuticals Ltd, ethanol was purchased from Merck. All the chemicals of analytical grade were used for the proposed study.

Methods

Characterization of drug

The drug can be characterized by determining its melting point using capillary fusion method. Fourier Transform Infrared (FTIR) spectroscopy was employed to confirm the identity of the drug through spectral analysis, specifically by detecting characteristic absorption bands corresponding to its functional groups¹⁰.

Determination of melting point

The melting point of the drug was assessed employing the capillary fusion technique to evaluate its thermal characteristics. In this method capillary tube was fused from one side and then filled with the drug (Levodopa) from another side. After that it was inserted into the melting point apparatus. Temperature was noted at which solid drug converts into liquid form by visual observation¹¹.

FTIR spectroscopy

FTIR spectroscopy is a widely utilized analytical technique that measures the absorption of infrared radiation by a sample to generate a spectrum, which provides information about the presence of specific functional groups and the molecular structure of the compound. In this study, the FTIR spectrum of pure levodopa was obtained using a SHIMADZU FTIR spectrophotometer (Japan). A suitable quantity of potassium bromide (KBr) was finely ground with the drug sample in an agate mortar to ensure homogenous mixing. Approximately 100 mg of the prepared mixture was compressed into a transparent pellet using a hydraulic press at a pressure of 600 kg. Spectral data were collected over the range of 4000 to 400 cm^{-1} with a resolution of 2 cm^{-1} . FTIR analysis served as a key method for structural elucidation and molecular

confirmation of Levodopa. In combination with melting point determination, these techniques were employed to verify the identity of the drug¹².

Analytical experimental method

Preparation of standard solution

Preparation of saline phosphate buffer pH 6.4

Accurately weighed 7.02g of sodium chloride, 1.36g of potassium dihydrogen phosphate and 1.79g of disodium hydrogen phosphate and dissolved in water. The volume was made up to 1000ml in volumetric flask¹³.

Preparation of working drug solution

The standard Levodopa (10mg) was accurately weighed and transferred into the (100ml) Volumetric Flask. The drug was dissolved properly in 100ml of saline phosphate buffer pH 6.4. to achieve a final concentration of 100µg/mL.

Determination of wavelength of maximum absorbance ($\lambda_{\max}$)

Appropriate volume 0.5ml of standard stock solution of levodopa was transferred into a 10 ml volumetric flask, and diluted to make the volume 10ml with saline phosphate buffer Ph 6.4. to give concentration of 5µg/ml. The solution was sonicated to remove air bubbles and scanned in the UV range 400–200 nm with a buffer as a blank. After acquiring the spectrum, $\lambda_{\max}$ was identified. The above method was repeated thrice¹⁴.

Preparation of calibration curve/ standard plot

The calibration curve was prepared by using Stock-1 to accomplish the six-diverse calibration standard representing 1, 2, 3, 4, 5, 6 µg/mL strength and sonicated. An absorbance of every calibration standard was estimated at $\lambda_{\max}$ 280 nm using fixed wavelength measurement mode¹⁵. The calibration curves representing concentration vs. absorbance was plotted utilizing in Microsoft Excel. Previously mentioned technique was recapitulated multiple time with the goal that reproducible outcomes can be obtained.

Analytical Method Validation

According to ICH guidelines, validation refers to the process of generating documented evidence that confirms, with a high level of confidence, that a specific procedure, process, or method consistently yields outcomes or products that meet predetermined specifications and quality standards. For the present study, various parameters were assessed to validate the analytical method, utilizing a set of predefined calibration standards, as outlined below¹⁶.

Linearity and range

The linearity of the developed UV spectrophotometric method was determined by analyzing six distinct calibration standards at varying concentrations¹⁷. Calibration curves were constructed by plotting absorbance against concentration, followed by evaluation using linear least squares regression analysis. The coefficient of determination (R^2 value) served as a key indicator to confirm the linear relationship between

the variables. The concentration ranges over which the method exhibited satisfactory linearity was defined as the analytical range of the proposed UV method¹⁸.

Accuracy

The accuracy of the proposed UV method was evaluated using recovery studies¹⁹. To the pre

analysed sample solutions, a known amount of standard stock solution was added at different levels, i.e. 80%, 100%, and 120%. The solutions were reanalysed by the proposed method. For calculating the percent recovery, following equation was utilized.

$$\%RC = (SPS - S / SP) \times 100$$

Where, % RC = Percent recovery

SPS = Amount found in the spiked sample

SP = Amount added to the sample

S = Amount found in the sample

Intra-Day Precision and Inter-Day Precision

Precision of the assay method was assessed in terms of repeatability by carrying out three independent assays of levodopa test arrangement and the % RSD of measurement (intra-day)²⁰. Intermediate precision of the method was checked by performing same methodology on three consecutive days (inter-day)^{21,22}.

Repeatability

Repeatability was determined by analysing a specific working concentration of Levodopa solution for six times²³.

Sensitivity

Limit of Quantification (LOQ)

In UV method development LOQ²⁴ was determined by utilizing the following equation.

$$LOQ = 10 \times SD / S$$

Where, S= slope

SD= Standard deviation of Y-intercepts

Limit of Detection (LOD)

In UV method development LOD was determined by utilizing the following equation.

$$LOD = 3.3 \times SD / S$$

Where, SD= Standard deviation of Y-intercepts

S= Slope

Robustness

Robustness of an analytical method refers to its capacity to maintain consistent performance despite small, intentional variations in method²⁵. Robustness was determined by analysing the levodopa samples by two distinct instruments.

Ruggedness

Ruggedness, the UV/VIS method was carried out by analysing triplicate sample of Levodopa by different analysts²⁶.

RESULTS AND DISCUSSION

The present study was structured to identify the drug and to develop a straightforward, rapid, precise, and reliable analytical method for its validation. Characterization of the drug was conducted through melting point analysis and interpretation of FTIR spectra. Quantitative estimation of Levodopa was subsequently performed using a UV-Visible double-beam spectrophotometer, measuring absorbance at its maximum wavelength ($\lambda_{\max}$) of 280 nm under simulated nasal conditions. The results obtained complied with the established acceptance criteria,

demonstrating that the method is highly suitable for the accurate determination of Levodopa in its bulk form. Saline phosphate buffer was used to mimic the nasal environment.

Confirmation of drug

Melting Point

By the capillary fusion method, the average range of melting point of the drug was found to be 277°C which corresponds to its actual melting range i.e. 276 –278 °C.

FTIR spectroscopy

By the interpretation of spectra which showed the presence of all functional groups of drug and confirmed the drug and its purity. The FTIR spectra is projected in the figure 2 and the various functional groups are given in table 1 for the drug levodopa.

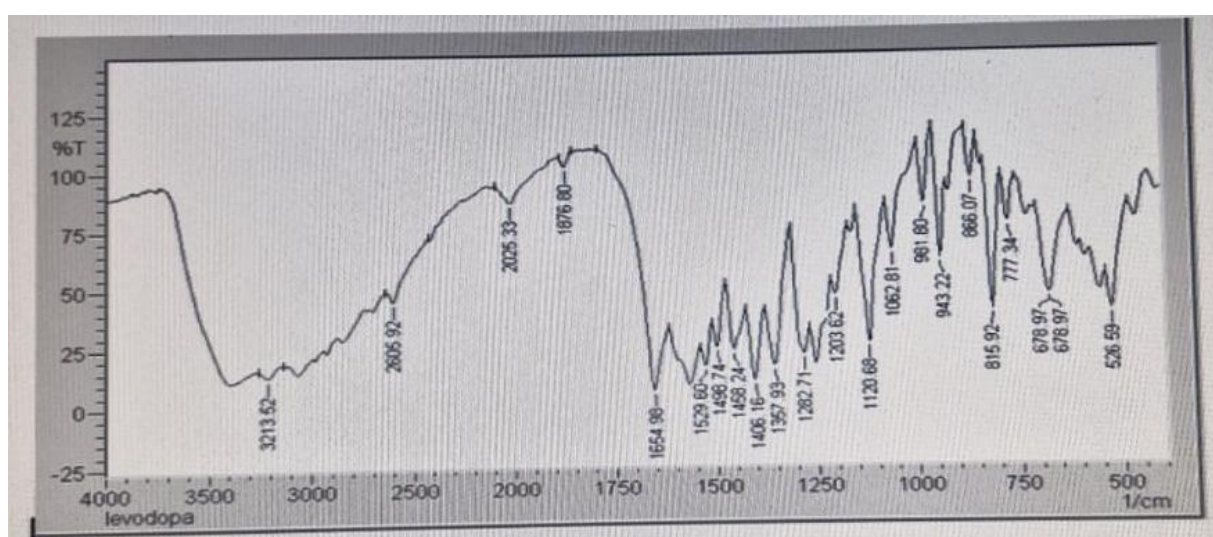


Figure 2: Fourier Transform Infrared (FTIR) spectra of drug levodopa.

Table 1: Major IR Peaks of Levodopa

Sr. No	Functional Group	Functional Group Range (cm ⁻¹)	Obtained Data(cm ⁻¹)
1	C-C Stretching	1000 – 1300 cm ⁻¹	1120.88 cm ⁻¹
2	C=C Stretching	1400 – 1600 cm ⁻¹	1529.60 cm ⁻¹
3	C=O Stretching	1700 – 1900 cm ⁻¹	1876.80 cm ⁻¹
4	N-H Stretching	3100 – 3500 cm ⁻¹	3213.52 cm ⁻¹
5	O-H Stretching	2500 – 3300 cm ⁻¹	2606.92 cm ⁻¹

Method development and optimization

Identification of wavelength of maximum absorbance is prerequisite for quantitative UV analysis. Solution representing absorbance value less than 1 is generally considered to be suitable for the determination of wavelength of maximum absorbance. Considering the prerequisite and the suitability, determination of maximum wavelength for levodopa solution (5µg/mL) was carried out using full scan mode of UV-Visible

spectrophotometer and the $\lambda_{\max}$ was found to be 280nm for Levodopa, the spectra is revealed in figure 3.

Analytical method validation

The method was validated for many parameters like one-dimensionality, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ)²⁷, robustness, ruggedness. Saline phosphate buffer was used to mimic the nasal environment.

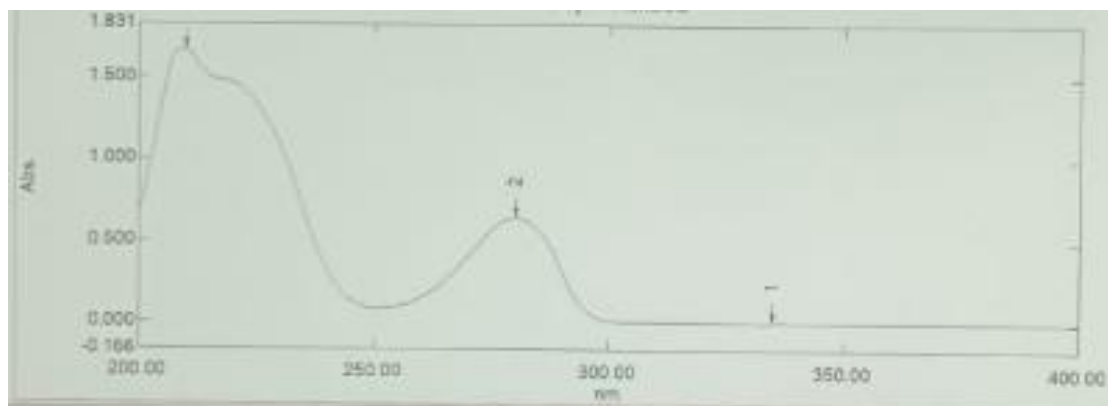


Figure 3: Spectrum Overlay Graph of Levodopa at 280nm.

Linearity and range

Different aliquots of levodopa in the range 0.1–0.6 ml were transferred into series of 10 ml volumetric flasks, and the volume was made up to the mark with saline phosphate buffer pH 6.4. to get concentrations 1, 2, 3, 4, 5, and 6 µg/ml, respectively. The solutions were scanned on a spectrophotometer in the UV range 200–400 nm. The spectrum was recorded at 280nm. The calibration plot was constructed as concentration vs. absorbance (Table 2 and Figure 4).

In UV-Vis spectrophotometry, Beer's Law linearity is crucial because it establishes a direct proportional relationship between the absorbance of a solution and the concentration of the absorbing substance, assuming a constant path length. This linearity allows for accurate quantitative analysis by enabling the determination of unknown concentrations based on measured absorbance values.

The Beer-Lambert Law, also known as Beer's Law, states that the absorbance (A) of a solution is directly proportional to the concentration (c) of the absorbing substance and the path length (b) of the light beam through the solution. Mathematically, it's expressed as: $A = \epsilon bc$ where ϵ is the molar absorptivity (or molar extinction coefficient), a constant that is characteristic of the substance at a specific wavelength.

If Beer's Law holds true (i.e., the relationship between absorbance and concentration is linear), it allows for

the determination of unknown concentrations of a substance by measuring its absorbance at a known wavelength. When analyzing a series of solutions with known concentrations, a plot of absorbance versus concentration should ideally be a straight line if Beer's Law is obeyed. This linear relationship is used to create calibration curves, which are then used to determine the concentration of unknown samples. Absorbance linearity is an indicator of the spectrometer's measurement accuracy. Deviations from linearity can indicate problems with the instrument or the sample, such as stray light or sample interactions.

While Beer's Law is a useful model, it's important to note that deviations from linearity can occur under certain conditions. At high concentrations, molecules can interact with each other, affecting their ability to absorb light in a predictable manner. If stray light reaches the detector, it can lead to an underestimation of absorbance, particularly at higher concentrations. Changes in the chemical environment, such as pH or ionic strength, can also affect absorbance and cause deviations from linearity. Factors related to instrument like the spectral bandwidth of the instrument and the quality of the optical components can also contribute to deviations. Thus, Beer's Law linearity is a cornerstone of UV-Vis spectrophotometry, enabling accurate concentration measurements. Understanding the conditions under which Beer's Law holds true, and being aware of potential deviations, is crucial for obtaining reliable results in quantitative analysis.

Table 2. Standard calibration curve of levodopa

Conc (µg/ml)	Absorbance			Mean	SD	%RSD
	n=1	n=2	n=3			
1	0.256	0.243	0.238	0.246	0.009	3.782
2	0.412	0.376	0.357	0.382	0.028	7.319
3	0.596	0.567	0.512	0.558	0.043	7.642
4	0.785	0.74	0.736	0.754	0.027	3.610
5	0.94	0.903	0.884	0.909	0.028	3.133
6	1.099	1.069	1.039	1.069	0.030	2.806

SD-standard deviation; RSD-relative standard deviation

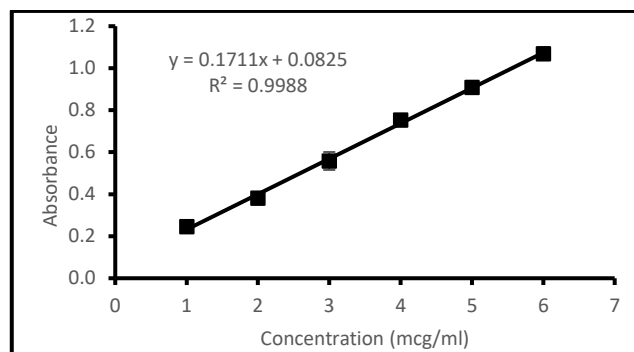


Figure 4. Standard calibration curve of levodopa at wavelength 280nm

Repeatability

Repeatability was determined by analysing the working concentration (4µg/ml) levodopa solution for six times. Repeatability in UV spectroscopy is a measure of how consistent measurements are under identical conditions. Factors that can influence repeatability are instrument stability including light source and detector; sample preparation, wherein accurate and consistent sample preparations can minimise the variations in sample concentrations; cell positioning, the positioning of the cuvette or sample cell in the spectrophotometer is crucial as it can affect light path and absorbance measurements; environmental conditions, fluctuations in temperature and humidity can impact the measurements.

Table 3: Precision results showing repeatability

Concentration (µg/ml)	Absorbance	Statistical Analysis
4	0.780	Mean = 0.756 (n=6)
4	0.740	SD = 0.017
4	0.736	% RSD = 2.207
4	0.767	
4	0.763	
4	0.759	

The repeatability parameter is typically expressed as %RSD, with a lower value indicating better repeatability. Ensuring good repeatability is vital for reliable analytical results and method validation. Table 3

describes the results obtained from repeatability studies that guarantee the precision of the method. %RSD was close to two.

Intra-day Precision and Inter-Day Precision:

Precision of the method was studied as intraday and interday variations. Intraday precision was determined by analysing the 3,4 and 5µg/ml of levodopa solutions for three times in the same day. Interday precision was determined by analysing the 3, 4 and 5µg/ml of levodopa solutions daily for three days over the period of week and the result varied for Interday precision. Table 4 and table 5 reflects the precision result for intraday and interday respectively. %RSD was close to two for all the concentration.

In UV-Vis spectroscopy, precision, along with accuracy, is a key parameter for validating analytical methods and ensuring reliable results for quantitative analysis. It reflects the reproducibility of measurements, indicating how closely results agree when the same sample is measured repeatedly under the same conditions. High precision suggests minimal random errors and contributes to the overall trustworthiness of the data indicating that the method produces consistent results, regardless of minor variations in experimental conditions. Precise measurements mean that the data points are tightly clustered around the mean value, leading to more reliable interpretations and conclusions. Precise measurements allow for the detection of subtle changes in the analyte concentration or other parameters. Precision is essential for quality control purposes, ensuring that products or processes consistently meet predefined standards.

Instrumental factors that influence precision are the quality and stability of the spectrophotometer, including its wavelength accuracy, spectral bandwidth, and photometric noise. Proper sample preparation, including consistent dilutions, clean cuvettes, and appropriate storage conditions, is essential for achieving high precision. Environmental factors that impact precision are temperature, humidity, and vibrations particularly for sensitive samples. Method Parameters like scan speed, data resolution, and the number of scans can influence the precision of the results.

Table 4: Intra-day Precision

Conc (µg/ml)	Morning	Afternoon	Evening	Mean	SD	%RSD
3	0.562	0.549	0.552	0.554	0.007	1.229
4	0.729	0.733	0.740	0.734	0.006	0.759
5	0.917	0.925	0.919	0.920	0.004	0.452

Table 5: Inter-day Precision.

Conc (µg/ml)	Day 1	Day 2	Day 3	Mean	SD	%RSD
3	0.538	0.510	0.517	0.521	0.015	2.797
4	0.742	0.714	0.706	0.720	0.019	2.625
5	0.929	0.901	0.895	0.908	0.018	1.999

Robustness

In UV-Vis spectrophotometry, robustness refers to the method's ability to remain unaffected by small, deliberate variations in experimental parameters. This is crucial for ensuring the reliability and reproducibility of results in routine analysis. Robustness testing helps identify potential sources of error and ensures the method can be used confidently under various conditions.

Small changes in the selected wavelength (e.g., ± 2 nm) can be tested to see if they impact the results. The method's robustness can be assessed by analysing samples at slightly different temperatures. Minor changes in the solvent composition (e.g., using a slightly different solvent or adjusting the ratio of solvents) can be evaluated. Depending on the specific method, other parameters like pH, flow rate, or sonication time can also be tested for robustness.

A robust method provides reliable results even when minor deviations from the optimal conditions occur, ensuring the method can be used in different labs or by different operators. It ensures that the method will produce consistent results when performed under similar, but not identical, conditions. Robustness testing helps identify potential sources of error and allows for adjustments to be made to the method to minimize their impact. Many regulatory guidelines require demonstrating the robustness of analytical methods used in pharmaceutical and other regulated industries. Robustness testing is an integral part of the method development and validation process, helping to refine the method and ensure its suitability for its intended purpose.

Two different concentrations of levodopa were prepared and sample was analysed at λ_{max} 280nm for Levodopa content by two distinct instruments. The results of this parameter is exhibited in table 6

Table 6: Robustness results

Concentration (µg/ml)	Absorbance measured in first instrument	Absorbance measured in second instrument	Mean	SD	%RSD
4	0.710	0.721	0.716	0.008	1.087
5	0.889	0.876	0.883	0.009	1.042

Ruggedness

Ruggedness in a UV-Vis spectrophotometer refers to its ability to produce consistent and reliable results despite minor variations in experimental conditions. Key ruggedness parameters include wavelength accuracy, photometric accuracy (absorbance linearity), and photometric repeatability, which ensure the instrument's performance is stable and trustworthy. These parameters are crucial for method validation and ensure the method's suitability for its intended use, such as in quality control or research.

Wavelength accuracy ensures that the instrument measures the correct wavelength of light. Deviations can lead to inaccurate absorbance measurements and compromised results. Photometric accuracy (Absorbance Linearity) assesses the instrument's ability to provide accurate absorbance readings across a range of concentrations. A linear relationship between absorbance and concentration is essential for quantitative analysis. Photometric repeatability evaluates the instrument's ability to produce consistent absorbance measurements when analyzing the same

sample multiple times. High repeatability is vital for reliable and reproducible results. The spectral bandwidth of the instrument should be appropriate for the analysis, as it can affect the resolution and accuracy of measurements. The instrument should minimize stray light, which can interfere with absorbance measurements and lead to inaccuracies. High resolution is needed to distinguish between closely spaced absorption bands, particularly in complex samples. The instrument should maintain stable absorbance readings over time, minimizing photometric drift.

Testing these parameters during method validation ensures the analytical method is reliable and can be used with confidence. In quality control settings, ruggedness testing helps ensure that the instrument is performing within acceptable limits and that the analytical results are trustworthy. In research, ruggedness testing helps researchers to ensure that their results are accurate and reproducible, regardless of minor variations in experimental conditions. Many regulatory bodies mandate the validation of analytical

methods and the testing of instrument performance parameters, including ruggedness.

By ensuring the ruggedness of the UV-Vis spectrophotometer, researchers and analysts can have greater confidence in their results and ensure the reliability of their analytical methods.

To assess the ruggedness of the proposed UV method, Levodopa solution was analysed by two different analysts. The results of this parameters are presented in table 7.

Table 7: Ruggedness results

Concentration (µg/ml)	Absorbance measured by first analyst	Absorbance measured by second analyst	Mean	SD	%RSD
4	0.763	0.767	0.765	0.003	0.368
5	0.910	0.909	0.910	0.001	0.078

Sensitivity

In UV-Vis spectrophotometry, sensitivity refers to the instrument's ability to detect small differences in analyte concentration, which is often reflected in the steepness of the calibration curve's slope. A more sensitive spectrophotometer can detect lower concentrations of a substance, making it valuable for applications where sample amounts are limited or when dealing with very dilute solutions.

In most UV-Vis spectrometers, sensitivity is closely linked to the signal-to-noise ratio (SNR). A higher SNR indicates a stronger, clearer signal relative to background noise, leading to more reliable measurements. A steeper slope in the calibration curve (absorbance vs. concentration) signifies higher sensitivity. This means a small change in concentration results in a larger change in absorbance. The molar absorptivity (ϵ) from Beer-Lambert's Law is another indicator of sensitivity. A higher ϵ value suggests that the substance absorbs more light at a given concentration, implying better sensitivity. Different detectors, such as photomultiplier tubes (PMTs) and silicon photodiodes, have varying sensitivities. PMTs are generally more sensitive than silicon photodiodes. A narrower slit width in the spectrometer can improve resolution but may reduce light throughput, potentially decreasing sensitivity. The efficiency of the diffraction grating in dispersing light also affects sensitivity. Proper sample preparation, including concentration and homogeneity, is crucial for accurate and sensitive measurements. Using longer path length cuvettes can increase the absorbance of samples, particularly for dilute solutions, thereby improving sensitivity.

Sensitivity is critical when working with low concentrations of analytes, such as in environmental monitoring or trace analysis. When dealing with precious or limited samples (e.g., biological samples), a sensitive instrument allows for accurate measurements with minimal sample consumption. Sensitivity is directly related to the accuracy and precision of measurements. Higher sensitivity leads to more reliable results. There is often an inverse relationship between sensitivity and resolution. Increasing sensitivity

by broadening the slit width may slightly reduce resolution. Accurate wavelength selection is crucial for obtaining reliable absorbance measurements and ensuring sensitivity. Photometric noise and drift can significantly affect sensitivity, particularly in low-light conditions. In conclusion, sensitivity is a crucial parameter in UV-Vis spectrophotometry, influencing the ability to detect low concentrations and ensuring accurate, reliable measurements

LOQ AND LOD

LOQ and LOD values for the developed method were found to be 0.526 and 0.174 µg/ml respectively. These values assure sufficient sensitivity of the developed method.

Overall optical characteristics of the developed uv-vis spectrophotometric method to estimate levodopa in simulated nasal media i.e., phosphate buffer saline pH 6.4 is shown in table 8.

Table 8: Optical characteristics

Parameter	Result
λ_{max}	280 nm
Beers law range	1-6 µg/ml
Correlation coefficient	0.9988
Regression equation	$y = 0.1711x + 0.0825$
Slope	0.1711
Intercept	0.0825
Repeatability (4 µg/ml)	Absorbance Mean: 0.756
Precision (4 µg/ml)	Intraday= 0.734, Interday= 0.720
LOD	0.526 µg/ml
LOQ	0.174 µg/ml

CONCLUSION

The identity of Levodopa was confirmed through melting point determination and FTIR spectroscopy. The observed melting point of 277 °C aligned with the

standard melting range, supporting the authenticity of the drug. Interpretation of the FTIR spectra revealed characteristic functional group peaks, further validating the compound's identity. A precise, cost-effective, and user-friendly UV spectrophotometric method was subsequently developed for the quantitative analysis of Levodopa.

The method demonstrated excellent precision, as indicated by %RSD values for both intraday and interday measurements being below 3%, which falls within acceptable limits. Additionally, the method exhibited good ruggedness and robustness, with %RSD values for these parameters remaining under 2%. The limit of detection (LOD) and limit of quantification (LOQ) were calculated as 0.526 µg/mL and 0.174 µg/mL, respectively, confirming the method's sensitivity.

Assay results showed strong concordance with the labelled content, indicating no significant interference from excipients in the formulation. The analysis of Levodopa using this UV-based approach was found to be rapid, accurate, and economical, making it suitable for routine quality control applications. In accordance with ICH guidelines, the developed method meets validation criteria for accuracy, precision, linearity, and overall reliability in the estimation of Levodopa.

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