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DEVELOPMENT AND VALIDATION OF A SIMPLE AND RAPID UV SPECTROSCOPIC METHOD FOR ESTIMATION OF VERAPAMIL HYDROCHLORIDE IN TABLET FORMULATION AND DETERMINING ITS DERIVATIVES

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ABSTRACT

A novel, straightforward, and highly specific UV spectroscopic method has been developed for the accurate determination of Verapamil Hydrochloride in tablet formulations. The UV-visible spectrum was analyzed to identify the wavelength at which maximum absorbance occurs, which was found to be 234 nm for Verapamil Hydrochloride. The method was validated in accordance with ICH guidelines, demonstrating linearity over a concentration range of 10.27 to 30.81 µg/mL. The mean recovery rate of Verapamil Hydrochloride was calculated to be 100.28%. The correlation coefficient was close to 1, indicating strong linearity and precision. Robustness tests confirmed that the method remains reliable under varying experimental conditions. Furthermore, recovery studies indicated that excipients in the tablet formulation do not interfere with the assay. This method is simple, rapid, and can be readily implemented in routine quality control of pharmaceutical products.

KEYWORDS: UV Spectroscopy, Verapamil Hydrochloride, Method Validation, Quantification, Pharmaceutical Analysis.

I. INTRODUCTION

Verapamil Hydrochloride, chemically known as α-[3-[[2-(3,4-Dimethoxyphenyl) ethyl] methylamino] propyl]-3,4-dimethoxy-α-(1-methylethyl) benzeneacetonitrile hydrochloride, is

an effective therapeutic agent commonly prescribed for the management of hypertension, angina, and certain heart rhythm disorders such as atrial fibrillation and atrial flutter. It is a member of the calcium channel blocker class of medications, which work by relaxing the blood vessels, thereby lowering blood pressure and reducing the heart's workload through a decrease in heart rate and contraction strength. Beyond its primary cardiovascular applications, Verapamil has been studied for other off-label uses, including the prevention of migraines and the treatment of some types of tremors.

Spectroscopy, the study of the interaction between matter and electromagnetic radiation, began with the analysis of visible light and its dispersion into a spectrum by a prism. Over time, the scope of spectroscopy expanded to include the interaction of matter with a broad range of electromagnetic radiation. In contemporary scientific practice, spectroscopy refers to the measurement of radiation intensity as a function of wavelength or frequency, and it has become an indispensable analytical tool in various fields, particularly in pharmaceuticals.

This research aims to develop and validate a fast, precise, and robust UV spectroscopic method for the determination of Verapamil Hydrochloride in tablet dosage forms, assessing its suitability for routine quality control in pharmaceutical applications.

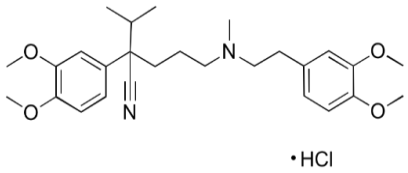

Figure 1: Verapamil Hydrochloride.

II. MATERIALS AND METHODS

Chemicals and Reagents

The Verapamil Hydrochloride active pharmaceutical ingredient (API) used in this study was generously provided by Chemclues Lifescience Pvt Ltd and served as the working standard. The Verapamil tablets used were purchased from local pharmacies. Methanol of AR grade was sourced from Finar Ltd., and purified water was obtained from a Millipore water purification system.

Instruments and Equipment

The analysis was conducted using a UV-Visible Spectrophotometer (Jasco, Model No. V-630) equipped with 1 cm path-length quartz cuvettes.

Diluent

The diluent used was a mixture of acetonitrile (ACN) and water in a 40:60 ratio.

Preparation of standard

A working standard of Verapamil Hydrochloride (21.87 mg) was transferred to a 50 mL volumetric flask (VF), to which 30 mL of the diluent was added. The solution was sonicated until the compound was fully dissolved, cooled, and then the volume was adjusted to 50 mL with the diluent.

From this stock solution, 2 mL was transferred to a 200 mL volumetric flask and the volume was completed with diluent.

Preparation of sample

Ten Verapamil Hydrochloride tablets were weighed and finely powdered. A quantity of this powder equivalent to the average weight of the active ingredient was transferred to a 250 mL volumetric flask, to which 170 mL of diluent was added. The mixture was sonicated for 30 minutes, with intermittent shaking, to ensure complete dissolution. The solution was then diluted with diluent to the 250 mL mark.

Subsequently, 1 mL of this solution was transferred to a 200 mL volumetric flask and the volume was completed with diluent.

III. METHOD DEVELOPMENT

To develop a robust and reliable UV spectrophotometric method for the quantitative analysis of Verapamil Hydrochloride, various parameters were optimized, including the choice of diluent, buffer, and buffer concentration. Several diluent compositions were tested to achieve optimal results, with degassed water being identified as the most effective. Consequently, water was selected as the final diluent for the analysis. The reference solution was scanned at a medium scanning speed over a UV range of 200–400 nm using the UV spectrophotometer, with the diluent used as the blank. After reviewing the spectrum, the wavelength of maximum absorbance (λmax) was determined. Verapamil Hydrochloride exhibited its λmax at 234 nm, which was confirmed as the final wavelength for analysis.

IV. METHOD VALIDATION

In accordance with the ICH Guidelines, the validation of this method was carried out to ensure its suitability for the intended purpose. The validation process assessed the

performance characteristics of the method, including its reliability, accuracy, and precision, to meet the necessary requirements for its intended use. The method was validated under the optimized experimental conditions using the appropriate instruments.

Specificity

To evaluate the specificity of the method, a solution containing a mixture of the tablet excipients was prepared following the sample preparation procedure. This was done to assess any potential interference from excipients. The spectral pattern of the test solution was compared to that of the reference solution, and the maxima (λ max) were found to correspond, confirming the absence of interfering peaks.

Linearity

Linearity refers to the ability of an analytical method to yield results that are directly proportional to the concentration of the analyte in the sample over a specified range. The linearity of Verapamil Hydrochloride was established by analyzing serial dilutions of a stock solution of the working standard. Five concentrations 2.19, 3.28, 4.37, 5.47, and 6.56 ppm were prepared and analyzed to verify the method's linear response.

Table 1: Linearity Concentration Levels of Chlorthalidone.

% level	*Volume of stock solution*	*Diluted to (ml)*	*Final concentration in ppm*
50%	1.0 ml	200	2.19
80%	1.5 ml	200	3.28
100%	2.0 ml	200	4.37
120%	2.5 ml	200	5.47
150%	3.0 ml	200	6.56

Accuracy

The accuracy of the method was assessed through recovery studies. Known quantities of the working standard were added to a fixed concentration of the pre-analyzed tablet sample, and the percentage recovery was calculated by comparing the area of the test solution with that of the pre-analyzed sample. Three sets of solutions were prepared at 50%, 100%, and 150% of the predefined concentration, and the percentage mean and individual recovery rates were calculated for each level.

Precision

Precision reflects the degree of consistency between multiple measurements of the same sample under identical conditions. To evaluate the repeatability of the method, six

independent assays of Verapamil Hydrochloride were performed. The mean area and percentage relative standard deviation (RSD) were determined, with an RSD of ≤ 2% being considered acceptable.

Intermediate Precision

Intermediate precision was assessed by conducting two separate repeatability trials on different days. The first set of trials was conducted as part of the repeatability assessment, while the second set was performed with a different analyst or using different instruments. The results from each day were compared by calculating the standard deviation, relative standard deviation, and mean value difference between the two sets of data.

Robustness

Robustness refers to the method's capacity to remain unaffected by small, deliberate changes in method parameters, indicating its reliability under typical operational conditions. The robustness of the method was evaluated by introducing variations in the sonication time and observing the effects on the results.

V. RESULTS AND DISCUSSION

The objective of the method validation was to confirm that the developed procedure is suitable for its intended use, as outlined in the ICH guidelines. The validation process assessed the method’s performance characteristics, which were expressed in terms of various analytical parameters, to ensure that the method meets the necessary criteria for its intended application.

Specificity

The specificity of the method was evaluated by comparing the UV spectra of the reference solution and the test solution. The spectral pattern of the test solution matched that of the reference solution, indicating that there were no interfering peaks from excipients. UV scans of the blank, placebo solution, standard solution, and sample solution are shown in Figures 2–5.

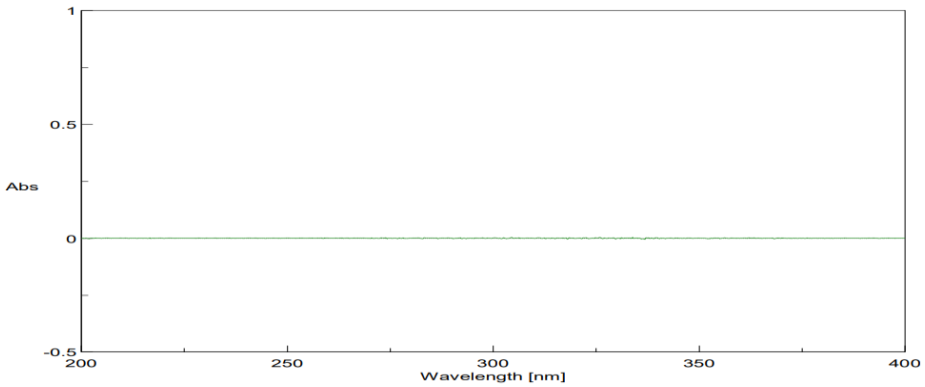

Figure 2: Spectrum of blank solution.

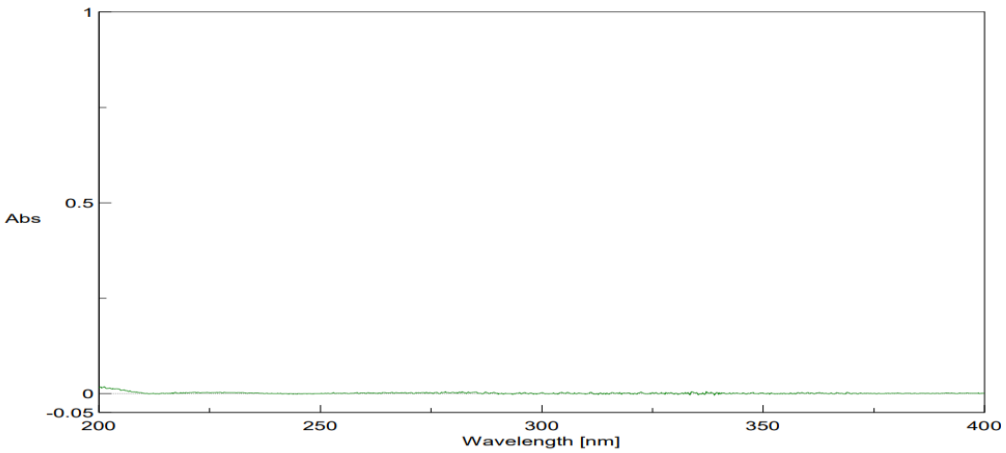

Figure 3: Spectrum of Placebo.

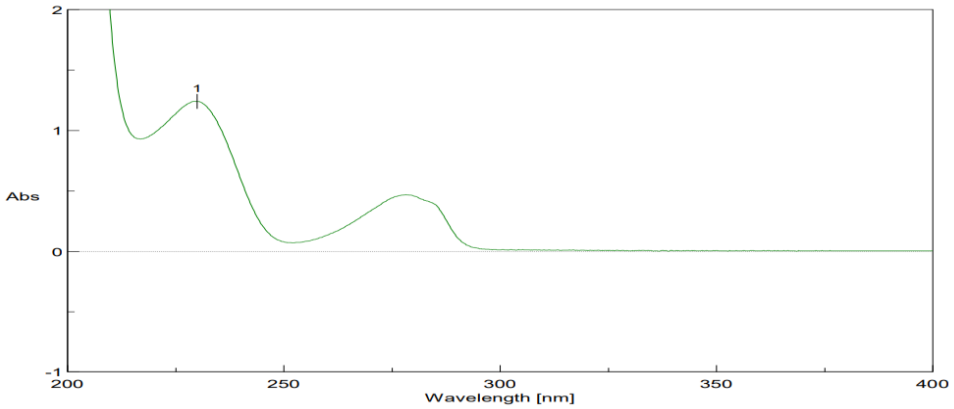

Figure 4: Spectrum of Standard Solution.

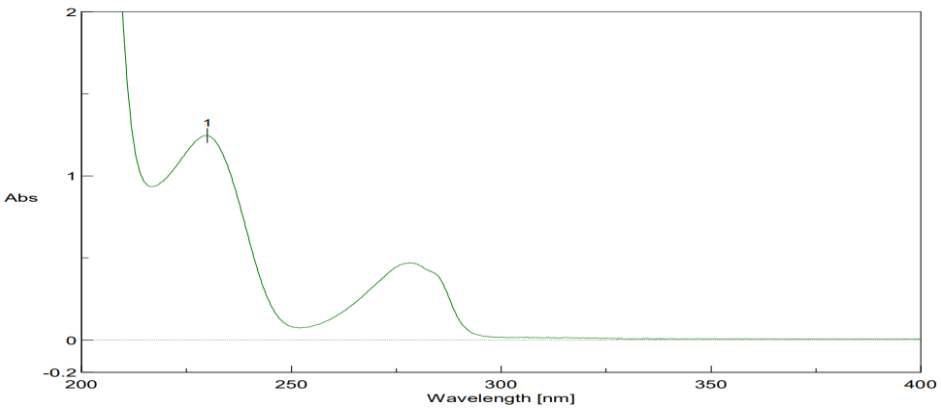

Figure 5: Spectrum of Sample Solution.

Linearity

To assess the linearity of the method, five different concentrations of Verapamil Hydrochloride (2.19, 3.28, 4.37, 5.47, and 6.56 µg/mL) were prepared. A plot of absorbance versus concentration was generated, as shown in Figure 6. Additionally, a residuals plot against concentration was created (Figure 7). The method demonstrated a linear relationship within the concentration range of 2.19–6.56 µg/mL (50–150% of the working concentration). The correlation coefficient (R) and the percentage of the Y-axis intercept met the acceptance criteria, as outlined in Table 2, confirming the linearity of the method.

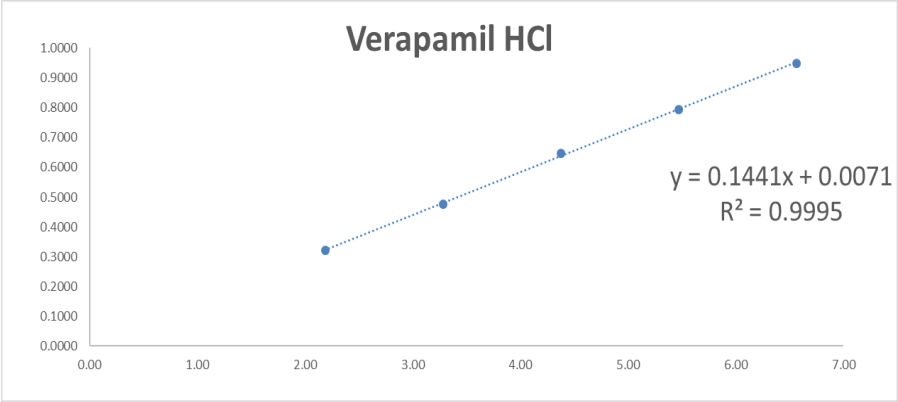

Figure 6: Linearity plot for Verapamil HCl.

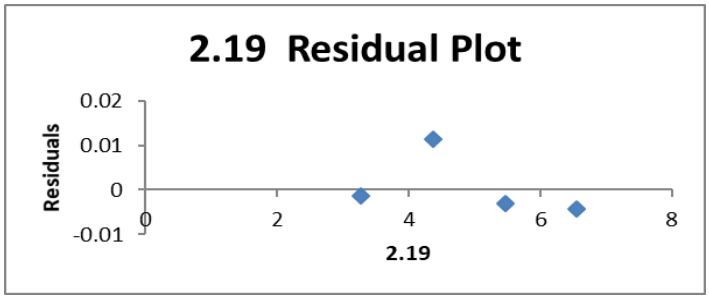

Figure 7: Plot of Residuals against concentration for Verapamil HCl.

Table 2: Observation table for linearity of Verapamil HCl.

Parameter for Linearity	*Values*	*Acceptance Criteria*
Correlation coefficient R	1.000	≥ 0.999
%Y – axis intercept	1.48	≤ ± 5 %
The slope of the regression line	0.14407	To be reported
Residual sum of squares	0.00016	To be reported

Accuracy

The accuracy of the method was evaluated by conducting recovery studies, where known quantities of Verapamil Hydrochloride were added to the pre-analyzed tablet sample. The percentage recovery results are summarized in Table 3. The method was considered accurate since the individual recovery values fell within the acceptance range of 97–103%, and the mean recovery was within the 98–102% range, meeting the validation criteria.

Table 3: Recovery at Different Concentration Levels.

Accuracy level	*% Recovery of Verapamil HCl*
50%	100.7
	100.9
	100.9
100%	99.6
	99.6
	99.6
150%	100.3
	100.5
	100.4
Mean recovery	100.28
Minimum recovery	0.64
Maximum recovery	0.63

Precision

The precision of the method was assessed by performing six independent determinations of Verapamil Hydrochloride. The results, shown in Table 4, indicated that the relative standard deviation (RSD) for all six measurements was well within the acceptance limit of ≤ 2%, confirming the method's repeatability and precision.

Table 4: Method Precision of Verapamil HCl.

Sample No.	*% Assay of Verapamil HCl*
Sample 01	100.5
Sample 02	100.5
Sample 03	100.6
Sample 04	100.6
Sample 05	100.5

Sample 06	100.5
Mean	**100.5**
STD Dev	**0.1**
% RSD	**0.1**

Intermediate Precision

Intermediate precision was evaluated by comparing the results from two separate repeatability experiments conducted on different days. The assay results for Verapamil Hydrochloride on Day 1 and Day 2 are presented in Tables 5 and 6, respectively. The comparison of the data from the two days demonstrated consistent performance, validating the intermediate precision of the method.

Table 5: Intermediate Precision of Verapamil HCl.

Sample No.	***% Assay of Verapamil HCl***
Sample 01	100.5
Sample 02	100.4
Sample 03	100.5
Sample 04	100.3
Sample 05	100.4
Sample 06	100.2
Mean	**100.4**
STD Dev	**0.1**
% RSD	**0.1**

Table 6: Comparison of two independent repeatability of Verapamil HCl.

Parameter	***1st-day Repeatability***	***2nd-day Repeatability***
Number of determinations	6	6
Mean (%) assay	100.5	100.4
RSD (%)	0.1	0.1
Mean value difference (%) **Acceptance Criteria:** < 2.0 % absolute	**0.1**	

Robustness

The robustness of the method was evaluated by intentionally varying method parameters, such as sonication time. The results showed that these deliberate changes did not significantly affect the method's performance, indicating that the method is robust. The data supporting these findings are shown in Table 7.

Table 7: Robustness Result for Verapamil HCl.

Parameter	**System suitability**		**% Assay**
	% RSD	**STD deviation**	
Sonication Time			

15 min	**0.1**	**0.1**	**100.6**
25 min	**0.1**	**0.1**	**100.4**

Solution stability

The stability of the standard and sample solutions was assessed over a 48-hour period. After 48 hours, the absorbance was measured, and no significant changes in the percentage assay were observed, indicating that the solutions remained stable. The results are provided in Table 8.

Table 8: Solution Stability Results for Verapamil HCl.

	Solution stability report of Standard		***Solution stability report of Sample***	
Stability condition	***% Assay***	***% Absolute Difference***	***% Assay***	***% Absolute Difference***
Initial	100.5	--	100.5	--
24 Hrs	100.5	0.0	100.5	0.0
48 Hrs	100.5	0.0	100.4	0.1

Filter compatibility

To assess the impact of filtration on the analytical results, the test solution was passed through different types of filter paper. The filter compatibility test demonstrated that the choice of filter did not interfere with the assay results. The findings are summarized in Table 9.

Table 9: Filter Compatibility Results for Verapamil HCl.

Filter Compatibility report of Verapamil HCl	
Filters	***% Assay***
Centrifuge	100.5
Nylon Filter	100.5
PVDF	100.6
PTFE	100.6

VI. DERIVATIVES

Zero Order

The zero-order spectrum is the initial step in derivative spectroscopy and serves as the baseline from which higher-order derivative spectra can be derived. The zero-order (D0) spectrum represents the normal absorption spectrum of the analyte, from which subsequent first, second, third, and fourth-order derivative spectra can be obtained. As the order of the derivative increases, the sensitivity of the analysis improves. In derivative spectroscopy, if a spectrum is expressed as absorbance (A) as a function of wavelength (λ), the derivative spectra are given by the following relations:

$A=f(\lambda), A=f(\lambda)$

First-order derivative spectrum

The first-order derivative spectrum is obtained by differentiating the zero-order spectrum once. It represents the rate of change of absorbance with respect to wavelength, and is plotted as:

$dA/d\lambda=f'(\lambda)$

The first-order derivative spectrum is more complex than the zero-order spectrum and passes through zero at the wavelength of maximum absorbance (λmax). This spectrum reveals positive and negative bands with maxima and minima. When scanning with a minimum and constant difference between two wavelengths, a dual-wavelength spectrophotometer can be used to obtain the first-derivative spectra. The first-order derivative spectrum is shown in Figure 8.

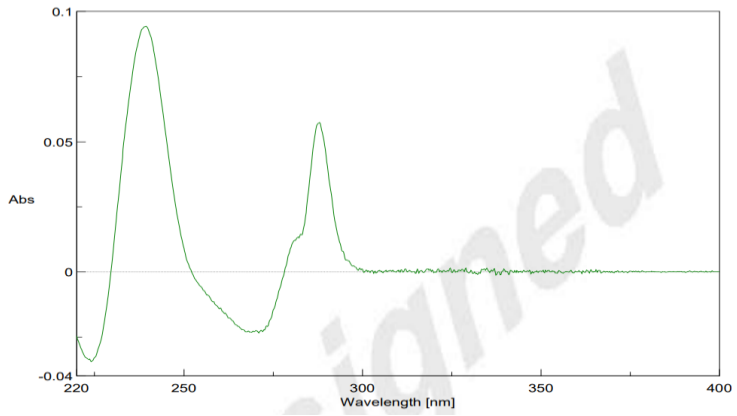

Figure 8: Spectrum of First Order Derivative for Verapamil HCl.

Second-order derivative spectrum

The second-order derivative spectrum is obtained by differentiating the absorbance spectrum twice. It provides a plot of the curvature of the absorption spectrum as a function of wavelength, given by:

$d^2A/d\lambda^2=f'(\lambda)$

The second derivative spectrum has a direct relationship with concentration, being directly proportional to the concentration of the analyte. A larger second derivative ratio indicates higher sensitivity. This method is particularly useful for obtaining atomic and molecular spectra. The second-order derivative spectrum is shown in Figure 9.

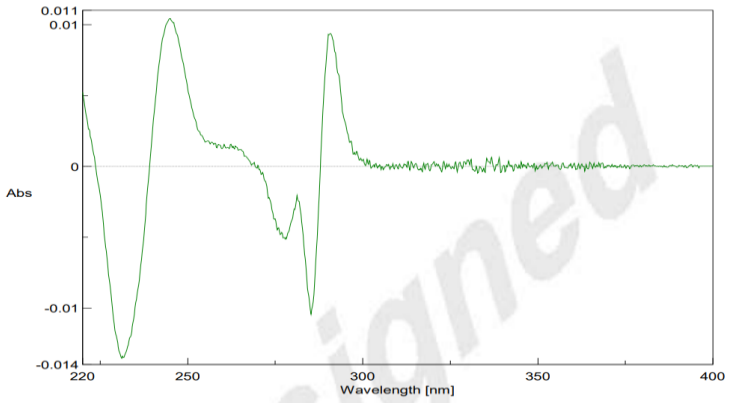

Figure 9: Spectrum of Second Order Derivative for Verapamil HCl.

Third-order derivative spectrum

The third-order derivative spectrum is obtained by differentiating the absorbance spectrum three times. It shows a more dispersed function compared to the original curve, plotted as: $d^3A/d\lambda^3=f'(\lambda)$.

The third-order spectrum is more complex and can be useful in distinguishing overlapping absorption bands. The third-order derivative spectrum is shown in Figure 10.

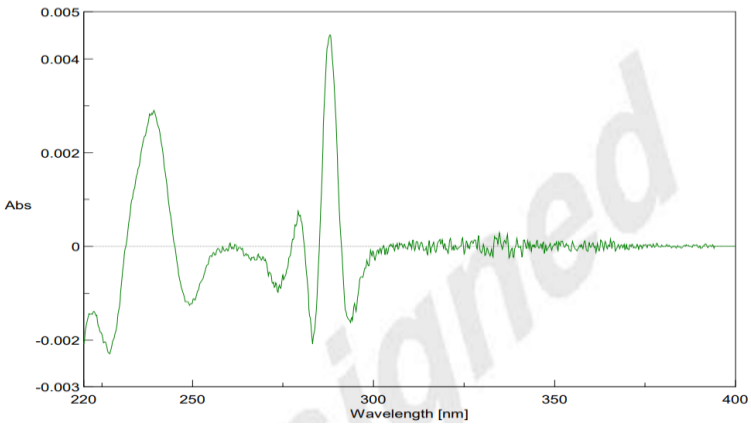

Figure 10: Spectrum of Third Order Derivative for Verapamil HCl.

Fourth-derivative spectrum

The fourth-order derivative spectrum is the result of differentiating the absorbance spectrum four times. It typically shows an inverted spectrum of the second-order derivative with sharper peaks at the center of the original absorption bands. This technique is especially useful for the selective determination of narrow bands, as it enhances the resolution of closely spaced absorption bands. The fourth-order derivative spectrum is plotted as: $d^4A/d\lambda^4=f'(\lambda)$.

The fourth-order derivative spectrum is shown in Figure 11.

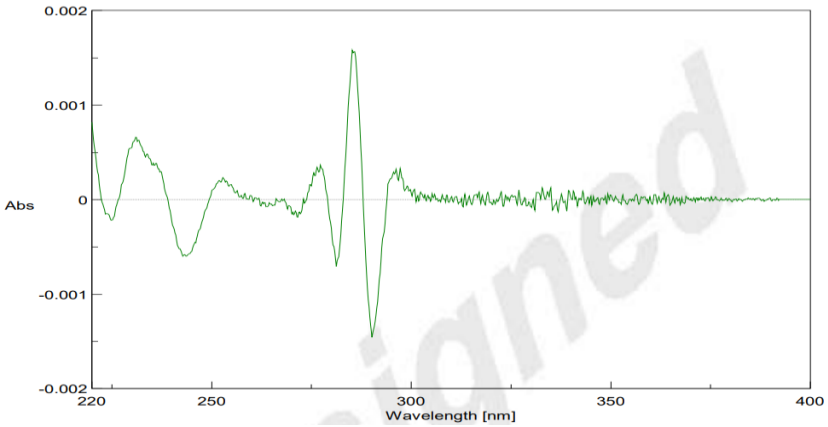

Figure 11: Spectrum of Fourth Order Derivative for Verapamil HCl.

Derivative spectroscopy enhances the extraction of detailed information from absorption spectra, aiding in quantitative analysis and improving sensitivity. Each order of the derivative provides unique insights into the molecular interactions of the analyte, making it a powerful tool for peak identification and the analysis of complex samples.

VII. ACKNOWLEDGEMENT

We would like to express our sincere gratitude to Chemclues Life Science Pvt. Ltd., Taloja, Navi Mumbai, India, for generously providing the API, standards, and laboratory facilities required for this research work.

VIII. CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding this research.

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