QUANTITATIVE UV SPECTROPHOTOMETRIC ANALYSIS OF PITAVASTATIN BULK DRUG AND PHARMACEUTICAL FORMULATIONS BY MULTIVARIATE CALIBRATION TECHNIQUE

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Abstract

This study set out to create a multivariate regression method-based Ultra Violet (UV) spectrophotometric assay that was simple, precise, sensitive, and validated for Pitavastatin analysis. Based on equations created using linear regression analysis and the associationbetween absorbance and concentration at five chosen equidistant wavelengths, thismultivariate calibration method was developed. A maximum wavelength of 266 nm was observed for Pitavastatin. For statistical significance testing, the results were examined. The result was a linear plot with a regression coefficient of 0.9993 for the concentration range of 3.5-6.5g/ml. There was a 0.1632 and 0.1759 percent RSD for intra-day and inter-day precision, respectively. The assay result was revealed to be 90%-101%w/w.

Keywords: Pitavastatin, HMG -CoA Reductase, UV spectrophotometry, Multivariate calibration, Assay, ICH guidelines.

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1. Introduction

Pitavastatin is also known by the brand names Itavastatin, Itabastatin, Nisvastatin, NK-104, and NKS-104 (3R,5S,6E) -7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quino-lyl]-3,5-dihydroxy- 6-heptenoate] having a molecular weight of 880.98, newly created statin (C50H46F2N2O8) is enantiomerically pure and entirely synthetic [1]. A new member of thefamily of statins known as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors is Pitavastatin [2]. Pitavastatin is a novel synthetic lipophilic statin that has beendemonstrated to lower serum levels of LDL-C and triglycerides and enchance high-density lipoprotein cholesterol (HDL-C) in dyslipidemic patients [3] . The dihydroxy pentanoic acidchain in pitavastatin makes it an enantiomer (3R,5S). Pitavastatin is categorized as a lipophilic medication [4]. Pitavastatin exhibits 1.6- and 3.5-fold higher affinities for HMG-CoA reductase than simvastatin or pravastatin, according to in vitro investigations,respectively [5]. Several analytical methods have been documented in this literature review forestimating pitavastatin, including UV-Spectroscopy[6][7], High-Performance Liquid Chromatography (HPLC)[8][9], High-Performance Thin layer Liquid Chromatography (HPTLC)[10], Ultra Performance Liquid Chromatography (UPLC)[11], Reverse Phase High-Performancee Liquid Chromatography(RP-HPLC) [12][13][14] and Liquid Chromatography-tandem Mass Spectrometry (LC MS/MS)[15] , among others.

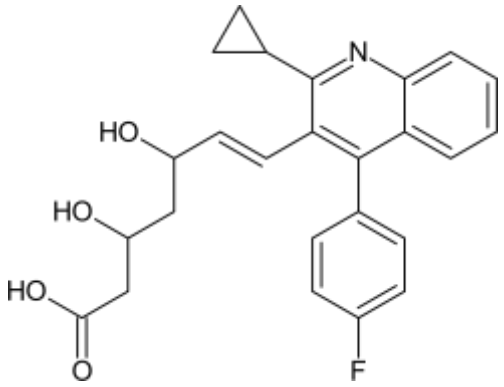

Figure 1. Chemical strucutre of Pitavastatin

The recommended method has been proved to be more accurate and precise than a conventional UV-Visible assay, which increases the level of confidence in the results because it assesses Pitavastatin directly. This method can be applied to bulk pharmaceuticals as well as different dosage forms and is more efficient, direct, and quick than previous approaches. This multivariate standardization method reduces the complexity of each individual result andturns it into a "m" value that serves as a dependent variable [16] . This analytical method would offer good sensitivity, resolving power, expediency, and cost-effectiveness for a validated measurement of Pitavastatin under ideal circumstances. Pitavastatin, an analyte (X), has own absorbance determined at 5 distinct wavelengths (245 nm, 247 nm, 249 nm, 251 nm, and 253 nm). Then, for any selected wavelength, the formula below can be followed. [16 – 21].

$A_{\lambda 245} = a\ X\ C_x + \ k_1$ ---------- (1)
$A_{\lambda 247} = b\ X\ C_x + \ k_2$ ---------- (2)
$A_{\lambda 249} = c\ X\ C_x + \ k_3$---------- (3)
$A_{\lambda 251} = d\ X\ C_x + \ k_4$---------- (4)
$A_{\lambda 253} = e\ X\ C_x + \ k_5$---------- (5)

Where A is the analyte's absorbance, a, b, c, d, and e are such linear regression function's slopes, k1, k2, k3, k4, and k5 are its intercepts at each of five predefined wavelengths, and Cx is its concentration. The chosen five equation systems (1–5) are shown below in short:

$$A_T = a\ X\ C_x + \ b\ X\ C_x + \ c\ X\ C_x + \ d\ X\ C_x + \ e\ X\ C_x + \ K_T \text{----------(6)}$$

The equation mentioned before can be reduced even more to

$$A_T = C_x\ (a + b + c + d + e) + \ K_T \text{---------- (7)}$$

Where AT and KT represent the entire sum of the intercepts of regression equations at the chosen five wavelengths, respectively. The formula below

is used to calculate the analyte X concentration.

$$C_x = \frac{A_T - K_T}{(a + b + c + d + e)}$$

1. Materials And Methods

Chemicals and reagents

- 0.1N Dilute hydrochloric acid (dil.HCL) (Gradient grade , Finar chemicals)

- Pitavastatin was obtained as a gift sample from Ideal Analytical and Research Institute, Pondicherry. The marketed tablet formulation used was Pivasta - 1 , Zydus Cardiva , India, (Label claim – 1 milligram Pitavastatin), acquired from a local market.

Instrumentation

- LAB INDIA 3092 UV-Visible double beam spectrophotometer

- Ultra Sonicator Bath

- Analytical balance

- Micropipette

Analytical Method Development

Determination of the solvent

In 0.1N dilute HCl, pitavastatin was found to be freely soluble. Hence, it was used for further dilutions of both standard and sample drug

Standard stock solution

Pitavastatin was dissolved in 10 ml of 0.1N diluted HCL to make a standard stock solution, and the same solvent was used to equilibrate the solution to the mark in a 10 mL standard flask. Transferring 0.5 mL of this solution to a second 10 mL volumetric flask and diluting the remaining solution to 10 mL allowed for a concentration of 10 g/mL. A range of concentrations (3.5-6.5 g/mL) of solution were prepared from this standard stock solution.

Determination of λmax

The standard stock solution was diluted in 0.1N diluted HCL to a concentration of 5 g/mL. This solution was evaluated in the Ultra-Violet region between 200 and 400 nm. 249 nm was found to be the maximum (Figure 2). The linear curve was created by plotting the absorbance versus the concentration (Table 1). The solutions were scanned over the region surrounding 249 nm, i.e., 245,247, 249, 251 and 253 nm, in order to strengthen the correlation and reduce instrumental oscillations.

Preparation of sample solution

Pitavastatin 20 mg tablets were accurately weighed and powdered. A weight equal to 10 mg was added to a volumetric flask with a volume of 10 ml, dissolved, and then diluted with methanol to the desired concentration of 1 mg/mL. This response was screened, and further investigation was conducted using it.

Method Validation

This method's sensitivity, precision, accuracy, and linearity have all been validated inaccordance with ICH Q2B requirements.

Linearity

From the usual stock solution of pitavastatin, various concentrations ranging from 3.5 to 6.5g/mL were created. These solutions were scanned throughout a wavelength range near their

respective absorbance maxima at 245, 247, 249, 251, and 253 nm in order to reduce instrumental fluctuations and improve the correlation. By drawing a concentration vs. absorbance graph, the absorbances were noted, and the standardizations were obtained. Figure 3 and Table 1.

Table 1- UV Calibration data at five distinct wavelengths

Concentration (µg/mL)	Absorbance				
	245 nm	247 nm	249 nm	251 nm	253 nm
3.5	0.298	0.314	0.321	0.318	0.306

4	0.341	0.360	0.368	0.367	0.349
4.5	0.384	0.406	0.414	0.410	0.393
5	0.428	0.451	0.461	0.455	0.436
5.5	0.471	0.497	0.508	0.501	0.480
6	0.514	0.543	0.554	0.547	0.523
6.5	0.558	0.588	0.601	0.598	0.561

#
Average of 7 determinations; UV= Ultra violet

By calculating the detection limit and quantification limit using the below formula, thesensitivity of the method was determined.

LOQ = 10 σ /S
..... (9)

Here, σ is the standard deviation (SD) of the lowermost concentration and S is the slope of the standard curve.

Precision
For the intra-day precision, scans of a 10 g/mL solution were carried out seven times during a brief period of time on one day, and seven times over seven distinct days for the inter-day precision.

LOD = 3.3 σ/S
..... (8)

Accuracy
The recovery study for the proposed method was resolved at 80%, 100%, and 120% with theuse of the conventional addition method, and the percentage recovery was calculated.

Assay
Pitavastatin concentration in tablet formulation was determined by measuring the extracted tablet solution's absorbance at 249 nm.

2. Result and Discussion

The λ_{max} of Pitavastatin was found to be 249 nm with 0.1N dilute HCL as the solvent asshown in Figure 2.

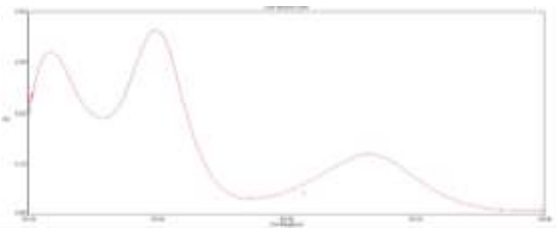

Figure 2-UV spectrum of Pitavastatin (10 µg/mL), λ_{max} at 249 nm

The method is linear within the specified concentration range of 3.5-6.5 g/mL. With R2= 0.9993-0.9997 for all of the calibration plots, the linear regression analysis demonstrates a strong linear relationship. The relative standard deviation percentage was found to be between 0.4800 and 0.7712 for accuracy. The obtained LOD and LOQ are 0.340 and 1.031 g/mL, respectively. As a result, it was determined that the results were within the validation parameter ranges of the ICH guidelines.

Linearity

The linearity was recorded at 245, 247, 249, 251 and 253 nm in the concentration range of 3.5-6.5 µg/mL and depicted in Figure 3 and corresponding calibration curves , residual plots are shown in the Figures 4 - 8 & 9-13 respectively. For each of the wavelengths, the low values of % relative standard deviation show that the technique is accurate and precise. LOD and LOQ were calibrated and reported in Table 2

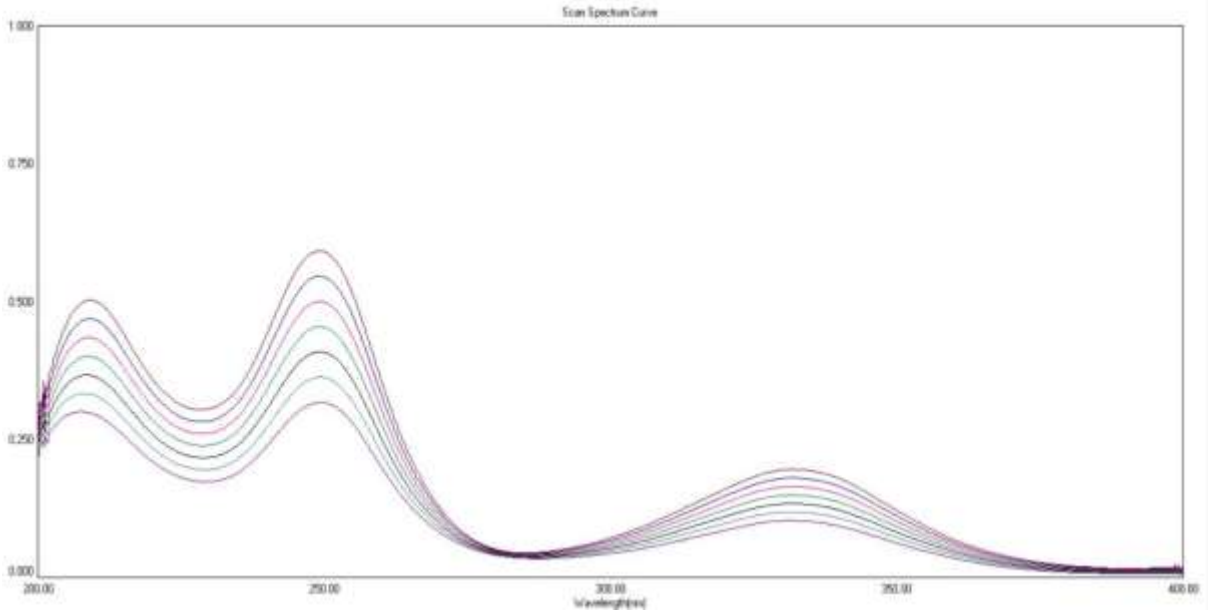

Figure 3- UV Spectrum of Pitavastatin showing linearity at 249nm

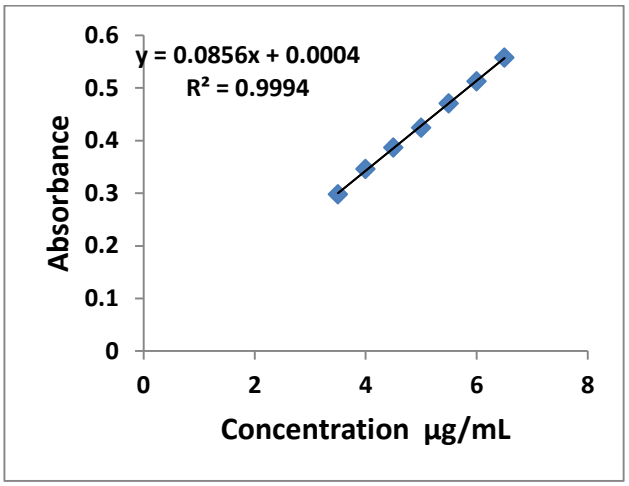

Figure 4- Calibration curve at 245nm

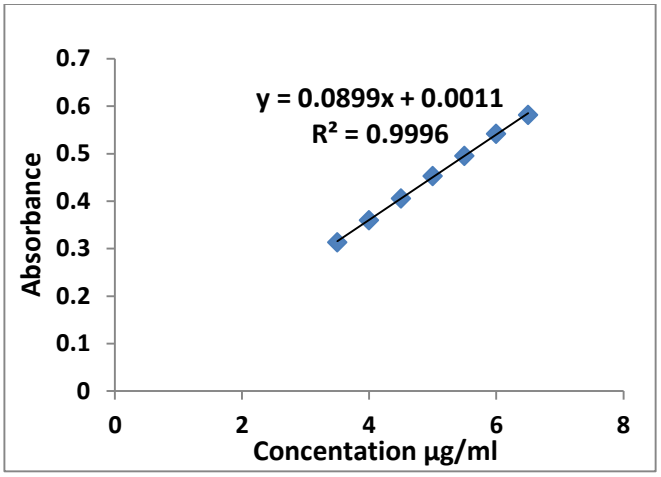

Figure 5-Calibration curve at 247 nm

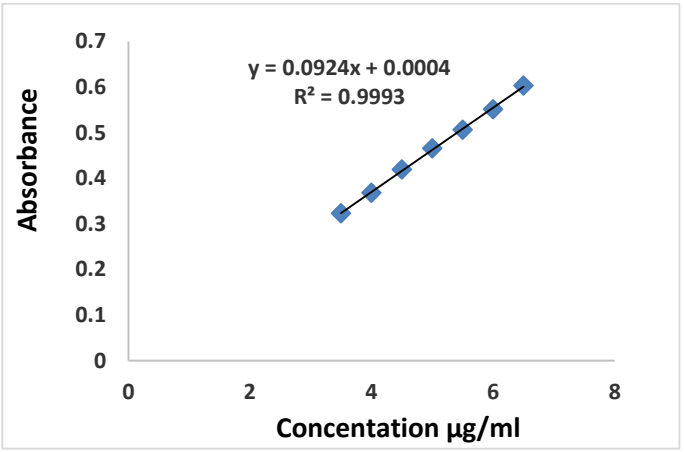

Figure 6-Calibration curve at 249 nm

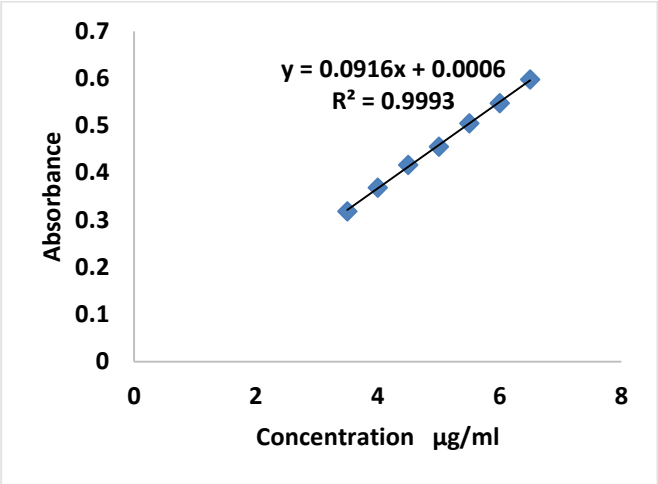

Figure 7-Calibration curve at 251 nm

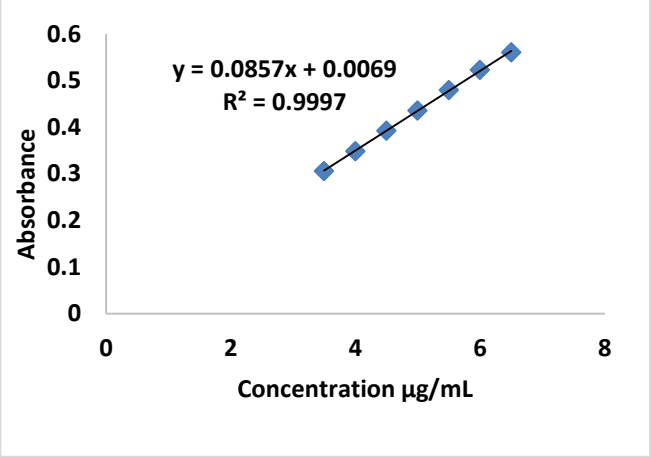

Figure 8-Calibration curve at 253 nm

Table 2: Linearity data with LOD and LOQ at selected five wavelengths

Wavelength (nm)	**Regression equation**	**R^2**	**LOD (µg/mL)**	**LOQ (µg/mL)**	**% RSD**
245	y = 0.0856x+0.0004	0.9994	0.253	0.767	0.57
247	y = 0.0899x +0.0011	0.9996	0.217	0.657	0.48
249	y= 0.0924x+0.0004	0.9993	0.312	0.944	0.64
251	y = 0.0916x+0.0006	0.9993	0.292	0.886	0.63
253	y = 0.0857x+0.0069	0.9997	0.187	0.568	0.43

*nm = Nanometer; µg/mL = Microgram per millilitre

Precision

The low standard deviation numbers show that this method is precise, and% RSD for the intra-day and inter-day precision were found to be respectively 0.1914 and 0.1979. It is less than 2% of the maximum at each wavelength. The approach is accurate and precise as seenby the low relative standard deviation percentage (Figure 14, 15).

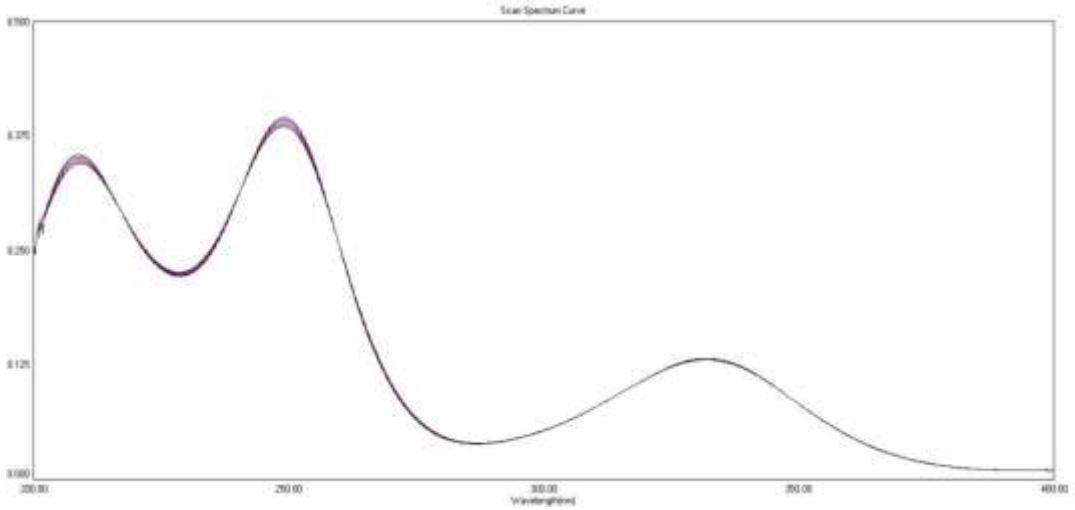

Figure 14-UV spectra showing intraday precision

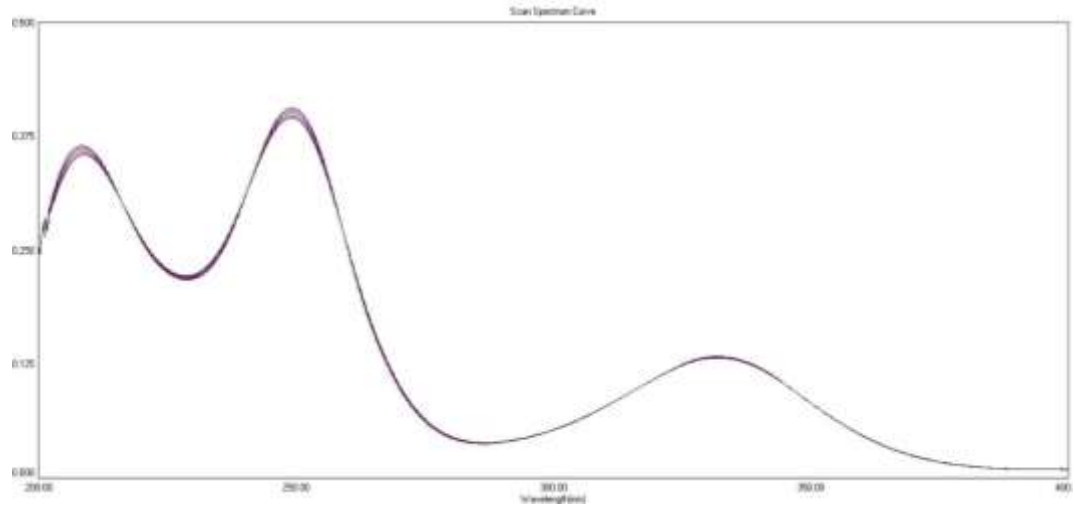

Figure 15-UV spectra showing interday precision

Recovery

The percentage recovery of pitavastatin was determined to be between 94% and 101.67 percent w/w, in accordance with ICH recommendations. The recovery fell within the permissible range of 90% to 101% weighted (Figure 16, Table 3).

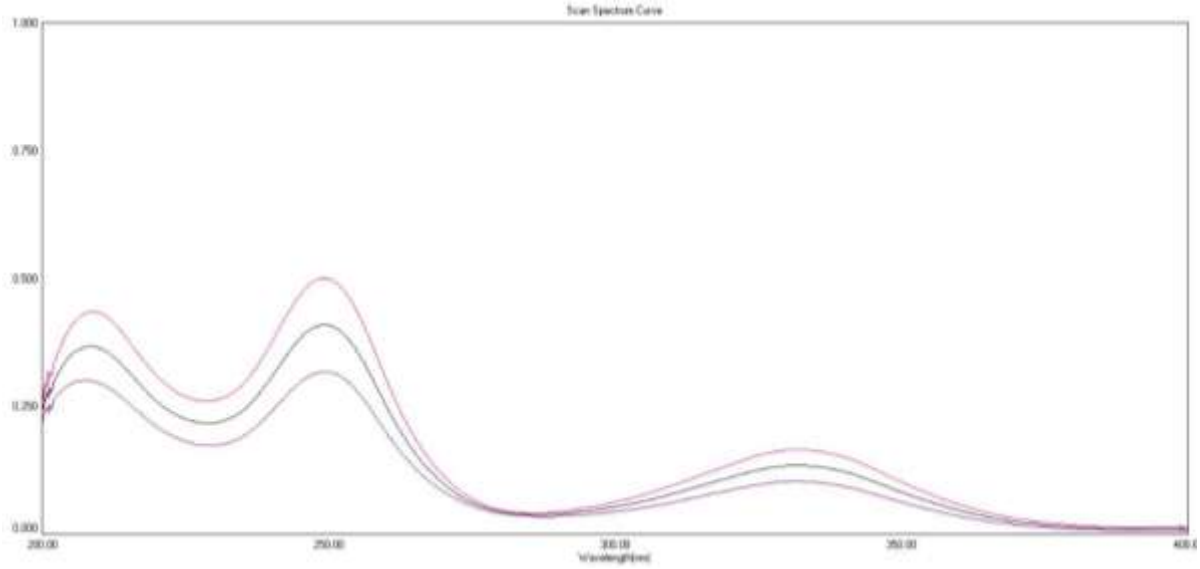

Figure 16-UV Spectrum showing accuracy of Pitavastatin

Table 3: Recovery Studies

Wavelength (nm)	**Amount present (µg/mL)**	**Amount added (µg/mL)**	**Absorbance**	**Amount recovered (µg/mL)**	**% Recovery**
245	2	2	0.385	3.9	97.50
		3	0.428	4.9	98.00
		4	0.514	6.08	101.33
247	2	2	0.396	3.9	97.50
		3	0.499	4.9	98.00
		4	0.589	5.9	98.33
249	2	2	0.401	3.8	95.00
		3	0.461	4.7	94.00
		4	0.592	5.9	98.33
251	2	2	0.397	3.9	97.00
		3	0.455	4.9	98.00
		4	0.547	6.1	101.67
253	2	2	0.386	3.9	97.50
		3	0.436	4.7	94.00
		4	0.523	5.8	96.67

Assay:

The tablet formulation's 249 nm UV absorbance was measured. The results of the assay reveal that the quantity and percentage are 0.98 mg and 98.00% w/w, respectively, with RSD values that are as shown in Table 4.

Table 4: Assay of Pitavastatin

Label claim (mg)	**Amount obtained (mg)**	**% Assay**
1	0.99	99.90
1	0.98	98.00
1	0.99	97.00
Average	0.98	98.00
SD		1.0000
% RSD		1.0204

3. Conclusion

For the pitavastatin assay, our unique multivariate technique is unquestionably more accurate,precise, repeatable, affordable, and sensitive than traditional UV-Visible Spectrophotometry. Pitavastatin is a medication that may be tested using this multilinear regression technique, as well as alternative dosage forms. Utilizing the ICH Quality Guidelines, this approach is validated and confirmed to fall within the predetermined validation limitations. As a result, it can be used for regular examination of Pitavastatin formulations in bulk drugs and pharmaceuticals as opposed to more expensive and complex procedures like HPLC and HPTLC.

List of symbols/abbreviations

nm = Nanometer

µg/mL = Microgram per millilitreg/mol = Gram per Mole

ICH = International Conference on Harmonization

UV = Ultraviolet

HPLC = High Performance Liquid Chromatography

HPTLC = High Performance Thin Layer Chromatography

Conflicts Of Interest

The authors report no conflict of interest in this study.

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