Method Development and Validation of Pitavastatin Calcium and its Degradation Behavior under varied Stress Conditions by UV Spectrophotometric methods

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ABSTRACT: UV spectrophotometric methods for the determination of pitavastatin calcium in pure and pharmaceutical dosage forms were developed and validated as per ICH guidelines. The standard pitavastatin calcium solutions were scanned between the ranges of 200-400 nm. The maximum absorbance of pitavastatin calcium in DMF (method A), HCl (method B) and NaOH (method C) was recorded at 266 nm. They obeyed Beers law concentration in the range of 10-45 µg/ml (method A), 0.25-2.0 µg/ml (method B) and 0.25-2.0 µg/ml (method C) with correlation coefficients 0.9996, 0.9998 and 0.9998 respectively. Stability study showed high stability of pitavastatin calcium in acidic, alkaline medium and at high temperature, but undergone degradation in oxidative stress condition. The developed methods were validated for linearity, precision, accuracy, LOD, LOQ, ruggedness, robustness and recovery studies. The proposed methods can be successfully used for the routine quality control analysis of pitavastatin calcium in bulk and commercial pharmaceutical formulations.

Key words: Pitavastatin calcium, UV spectroscopic method, acid, alkali, oxidative, thermal and UV degradation.

INTRODUCTION

Pitavastatin calcium (PTC) is a drug which comes under the category of statin group. It is chemically called monocalcium (3R,5S,6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolinyl]-3,5-dihydroxy-6-heptenoic acid with molar mass 880.98 g/mol. It is a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase[1]. It lowers both total cholesterol and low density lipoprotein (LDL) cholesterol in both animals and humans. Metabolism of it by the cytochrome P450 is smallest, reducing the risk of drug-drug interactions. Like all other statins, pitavastatin calcium is used for controlling hypercholesterolemia and for the prevention of cardiovascular disease.

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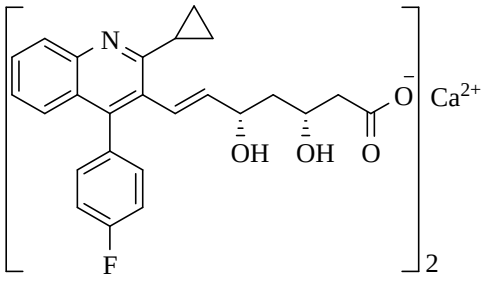


Pitavastatin calcium (PTC)

The chemical formula of PTC is $C_{50}H_{46}CaF_2N_2O_8$. PTC is odorless and looks like a white powder. It is hygroscopic in nature and very slightly unstable in sunlight. It is freely soluble in pyridine, chloroform, dilute HCl, DMSO and DMF. The literature review exposed several analytical methods for the determination of pitavastatin calcium by titrimetric[2], visible spectroscopic methods[3-6], stability indicating studies[7-9], fluorimetric method[10], chromatographic methods like HPTLC[11], RP-LC[12-13], HPLC[14], HPTLC[15] and LC-MS/MS[16-17], Electro analytical techniques.[18-19] The above reported

methods suffered from various disadvantages like heating step, slow reaction, extraction step, multi step reactions, tedious control of experimental variables and less sensitivity. To overcome the above problems, we developed accurate, reproducible and sensitive spectrophotometric methods for the analysis of pitavastatin calcium.

MATERIALS AND METHODS

Chemicals required. Pure API pitavastatin calcium (purity 99.5%) was gifted by Orchid Pharmaceuticals, Chennai and was used as received. Commercial tablet formulations were purchased from the local market namely, Pivasta 2 and Pivasta 4 manufactured by Zydus Cardiva, India (mfg date 12/2016; Exp date 11/2018 B No. S605629). Spectroscopy grade of N, N-dimethylformamide (DMF), hydrochloric acid (HCl), sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) were purchased from Merck.

Instrumentation. The three developed UV spectrophotometric method measurements were carried out using an Agilent 8453 model UV-VIS spectrophotometers with a diode array detector (DAD) in the range of 200-400 nm. The reference and sample solutions were recorded using 1 cm quartz cell. Shimadzu ATX 224 analytical balance was used for weighing purpose.

Standard drug solution. An accurately weighed 10 mg of pitavastatin calcium was dissolved initially in 10 ml DMF and finally made up to the mark with the same solvent in 100 ml calibrated flask whose concentration is 100 µg/ml. The stock solution was diluted to 50 µg/ml which was used for method A as a working concentration. In the same way, 10 µg/ml and 5 µg/ml PTC solutions were prepared for method B and method C. These solutions were stable for 1 week in the refrigerator when stored in an amber colored bottles.

Experimental Section

Procedure for calibration

Method A. Series dilutions of working concentration solutions (50 µg/ml) were made by taking 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 ml of PTC solution into 10 ml volumetric flasks and diluting to the volume with DMF to obtain the concentrations ranging from 10-45.0 µg/ml. The above solutions were scanned over the range 200-400 nm against DMF as blank. The maximum absorbance was found to be at 266 nm. The calibration plot was constructed by plotting concentration versus absorbance at 266 nm.

Method B and method C. Aliquots of working solution containing 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0 ml from 10 µg/ml for method B and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 ml from 5 µg/ml for method C pipetted out accurately into 10 ml of volumetric flasks and 1ml of 0.01 M HCl and NaOH was added to each flask and volume was made up to the mark with distilled water. The above solution was scanned in the range of 200-400 nm against with suitable blank solution. The absorbance of each solution was recorded and the wavelength was found to be 266 nm. The calibration curves were plotted by considering the absorbance versus the concentration of PTC solution.

Procedure for commercial tablets

Method A. Twenty tablets each containing 2 mg or 4 mg of PTC were weighed accurately and finely powdered using mortar and pestle. An amount of the powder equivalent to 10 mg of PTC was accurately weighed, transferred into a 100 ml volumetric flask and dissolved in 25ml of DMF. The contents were shaken thoroughly for about 15 min. After ultrasonic vibration for 15 min, the volume was diluted to the mark with DMF, mixed well and filtered using Whatman number-41 filter paper. First 10 ml portion of the filtrate was rejected and a suitable aliquot of the filtrate (containing 100 µg/ml PTC) was used as the stock solution. This solution was diluted to 50 µg/ml and used for the assay by the recommended procedure of method A and subjected to the analysis following the procedure described earlier.

Method B and C. Twenty tablets each containing 2 mg or 4 mg of PTC were weighed accurately and grounded into a fine powder by using mortar and pestle. PTC tablet powder equivalent to

10 mg of pure PTC was accurately weighed, which was initially dissolved in 25 ml of DMF and filtered using Whatman number-41 filter paper. The first 10 ml portion of the filtrate was discarded and the remaining filtrate were transferred into a 100 ml volumetric flask and made up the volume with distilled water (containing 100 µg/ml PTC) used as a stock solution. The above stock solution was diluted stepwise to get a working concentration 10 µg/ml PTC used for the method B and 5 µg/ml PTC for method C and subjected to analysis by following the procedure described earlier.

Method validation. The developed methods were validated linearity, sensitivity, precision, accuracy, robustness, ruggedness, selectivity, interference and recovery studies according to ICH guidelines[20].

Procedure for the selectivity study. Placebo blank is a mixture of commonly added excipients within formulations. Based on the amount of excipients present in a PTC tablet, a placebo blank of the composition consists of lactose monohydrate, hydroxypropylcellulose, hypromellose, magnesium alumina metasilicate, magnesium stearate, titanium dioxide, triethyl citrate and colloidal anhydrous silica was prepared and then subjected to analysis as described under "procedure for tablets", and then analysed using the procedure described under "procedure for calibration". A synthetic mixture was prepared by adding 10 mg of pure PTC to the above mentioned placebo blank and the mixture was homogenized. Following the same procedure for tablets, the synthetic mixture solution was prepared and a suitable quantity was subjected for the analysis by the three developed methods.

Degradation study

Acid and alkaline degradation. In acid and alkaline degradation, 5µg/ml of PTC solution was prepared in DMF. 4 ml of this PTC solution was pipetted out into 10 ml volumetric flasks and added 1.0, 2.0 ml of 0.01M HCl for acid degradation. This was heated for 30 minutes at 80°C in an oil bath. After 30 minutes the solution was brought to room temperature and finally diluted to the mark with distilled water and the corresponding absorbance values were recorded. The same procedure was repeated for alkaline degradation by using 0.01M NaOH. The obtained spectra were shown in figures 7 and 8.

Oxidative degradation. In hydrogen peroxide degradation studies, 5 µg/ml of PTC was prepared in distilled water which was used as a working solution. From that 0.5 ml and 1 ml of PTC solution was pipetted out and added 9 ml of 3 and 5% H_2O_2. Then, the solution was kept at room temperature for 30 minutes and diluted to the mark with distilled water. The absorption spectra of the resulting solutions were recorded which was shown in the figures 9 and 10.

Thermal degradation. To study the susceptibility of the drug under thermal stress conditions, the drug was spread in a glass petri-dish and placed in the oven maintained at 100°C for 1 hour. After bring to room temperature, 10 µg/ml of PTC solution was prepared in DMF and diluted up to the mark with the respective solvent. The absorption spectrum was recorded which was shown in figure 11.

UV degradation. UV degradation was carried out for exposing the drug in sunlight for 1 h. After brought to room temperature, 1mg of pure PTC was weighed initially dissolved in DMF and diluted up to the mark with the respective solvent in 100 ml volumetric flasks which was used as a stock solution. This stock solution was further diluted to 5 µg/ml. The absorbance was recorded which was shown in figure 12.

RESULTS AND DISCUSSION

Absorption spectra. An attempt was made to develop a simple, rapid, highly sensitive, precise and accurate analytical method for pitavastatin calcium in both pure and pharmaceutical preparations. The developed UV method allows rapid and economical evaluation of PTC in tablets without any time-consuming sample preparation. However, the spectrophotometric method involves simple instrumentation compared with other instrumental techniques. Further, PTC stock solutions and working

standards were made in DMF. It showed maximum absorption at 266 nm for methods A, B and C which were shown in figures 1, 2 and 3.

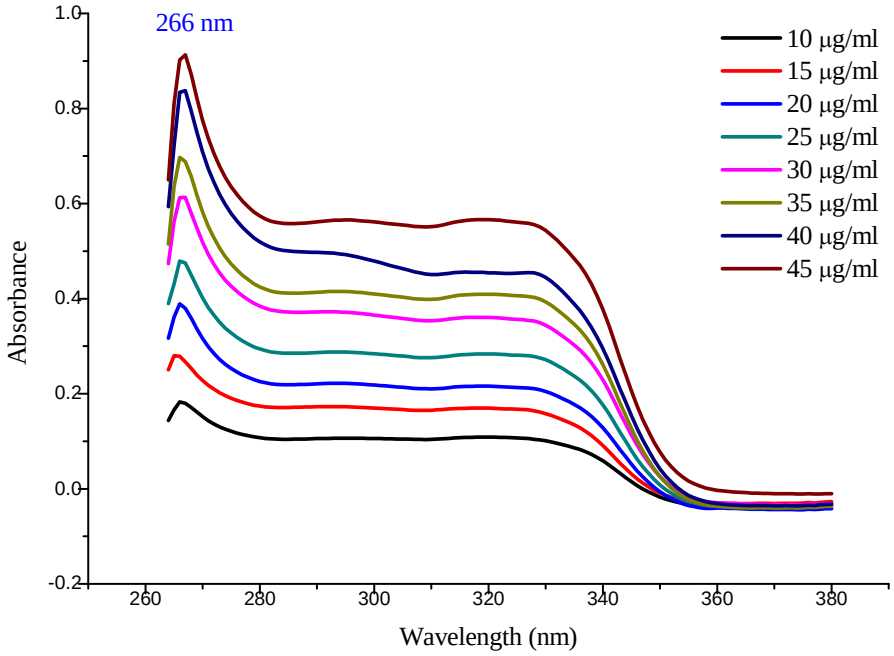


Figure 1. Absorption spectra of PTC in DMF at different concentrations (method A)

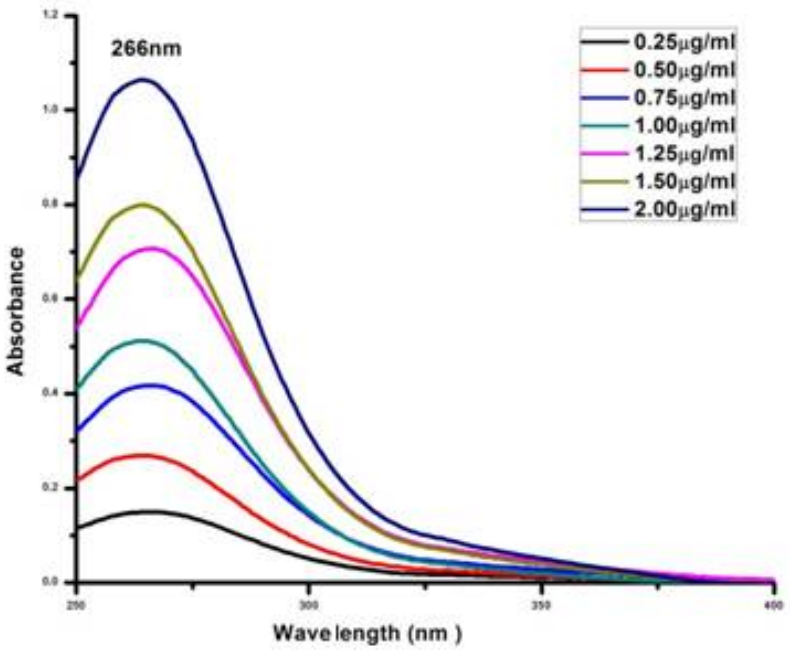


Figure 2. Absorption spectra of PTC in 0.01M HCl at different concentrations (method B)

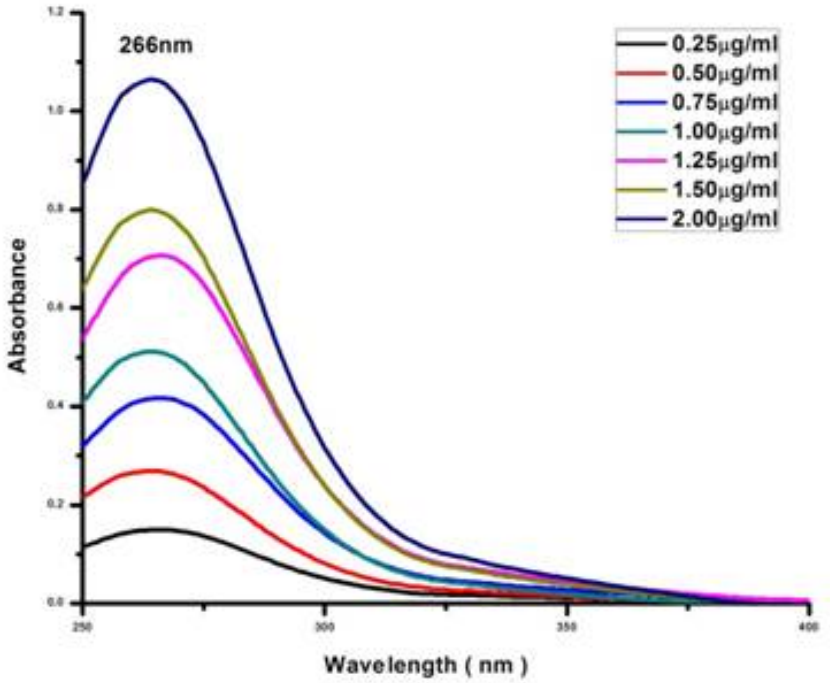


Figure 3. Absorption spectra of PTC in 0.01M NaOH at different concentrations (method C)

Calibration plot. The calibration curves for methods A, B and C were plotted over concentration versus absorbance which were shown in the figures 4, 5 and 6. The excellent linearity's of the calibration curves were experimentally proved by the high values of correlation coefficient (r) 0.9996 for method A, 0.9998 for both the methods B and C. From that, slope, intercept, LOD, LOQ, molar absorptivity, Sandell sensitivity, range, the standard deviation of intercept and standard deviation of slope values are calculated and described in table 1. The high values of molar absorptivity and very low values of Sandell sensitivity proved that the three developed methods were very sensitive.

Precision and accuracy studies. The developed methods were tested by taking both precision and accuracy in intra-day (n=5) and inter-day (n=6) at three different concentration levels within the working concentration limits. The concentration levels were 20, 25 and 30 µg/ml for method A, 0.5, 1.0, and 1.5 µg/ml for method B and C. The percentage relative standard deviation (% RSD) was calculated for the precision of the developed methods which was obtained as less than ≤1.78 %. The accuracy of the developed methods were calculated by the percent relative error (%RE) which was ≤ 1.30%. Therefore, the developed methods showed good accuracy and precision. The results of these studies were listed in table 3.

Robustness and ruggedness. The robustness was determined at three different concentration levels of PTC by a slight change in time for method A and in the volume of the reagents which were 0.75 and 1.0 ml for both methods B and C. The % RSD values for these changes were varied from 1.32% to 1.90% i.e less than 2% in both pure and pharmaceutical formulations whereas the ruggedness was carried out by three different analysts and also three different cuvettes by a single analyst. % RSD for both inter-analysts and inter-instruments was found to less than 2%. These low values of precision (Table 4) indicated that the robustness and ruggedness of the developed method were good.

Selectivity studies. In the analysis of placebo blank studies, there was no peak observed for all the developed method whereas in synthetic mixture analysis, the expected maximum wavelength was

resulted. The percentage recovery values ranged from 99.79-101.99 with the standard deviation values less than 1%. Thus, these results suggested that the added inactive ingredients did not interfere in the analysis of the developed methods which was shown in table 5.

Table 1. Summary of optical characteristics and validation parameters.

Parameter	Method-A	Method-B	Method-C
λ_{max}, nm	266	266	266
Beer's law limits, µg/ml	10-45	0.25-2.0	0.25-2.0
Molar absorptivity (ε), L mol^{-1} cm^{-1}	1.73×10^{4}	4.84×10^{4}	4.74×10^{5}
Sandell sensitivity, µg cm^{-2}	5.07×10^{-8}	1.81×10^{-9}	1.85×10^{-9}
Limit of detection, µg/ml	4.78	0.01	0.096
Limit of quantification, µg/ml	14.49	0.30	0.30
Regression equation, Y = a + bX			
Intercept (a)	-0.0250	0.0058	0.0023
Slope (b)	0.0207	0.5305	0.5544
Correlation coefficient	0.9996	0.9998	0.9998
Standard deviation of intercept (S_a)	0.0051	0.0038	0.0043
Standard deviation of slope (S_b)	0.0002	0.0032	0.0037

*Limit of determination as the weight in µg/ml of solution, which corresponds to an absorbance of A= 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm.

** Y=a +bX, where Y is the absorbance, a is the intercept, b is the slope.

Table 2. Summary of results of stress degradation studies.

Stress condition	Time	Observation	% Degradation
Acid	80ºC, 30 min	No degradation	0
Alkali	80ºC, 30 min	No degradation	0
3% H_2O_2 and 5% H_2O_2	30 min	λ_{max} shifted	7.14% and 9.77%
Thermal	100ºC, 1 h	No degradation	0
UV	Sunlight, 1 h	Slight degradation	0.75%

Table 3. Intra-day and inter-day precision and accuracy studies

Methods	PTC taken (µg/ml)	Intra-day (n=7)			Inter-day (n=5)		
		PTC found[a] (µg/ml)	% RSD[b]	% RE[c]	PTC found[a] (µg/ml)	% RSD[b]	% RE[c]
Method-A	20	20.14	1.14	0.70	20.18	1.39	0.90
	25	25.07	1.25	0.28	25.12	1.34	0.48
	30	30.19	1.60	0.63	30.15	1.72	0.50
Method-B	0.5	0.501	1.22	0.20	0.503	1.28	0.60
	1.0	1.005	1.44	0.50	1.006	1.49	0.60
	1.5	1.510	1.77	0.66	1.505	1.78	0.30
Method-C	0.5	0.502	1.58	0.40	0.504	1.60	0.80
	1.0	1.003	1.63	0.30	1.005	1.65	0.50
	1.5	1.520	1.68	1.30	1.510	1.70	0.67

[a]Mean value of five determinations , [b]Relative standard deviation (%) , [c]Relative error (%)

Table 4. Robustness and ruggedness.

Methods	PTC taken (µg/ml)	Method robustness	Method ruggedness	
		%RSD (n=3)	Inter-analyst (n=3)	Inter-instrument (n=3)
Method-A	20	1.32	1.39	1.50
	25	1.45	1.47	1.66
	30	1.80	1.98	1.88
Method-B	0.5	1.38	1.40	1.45
	1.0	1.44	1.53	1.65
	1.5	1.78	1.99	1.67
Method-C	0.5	1.78	1.80	1.83
	1.0	1.81	1.85	1.92
	1.5	1.90	1.84	1.95

HCl and NaOH volumes used were 0.75 and 1.25 ml for method B and C whereas in method A, time was changed.

Table 5. Analysis of synthetic mixture by the developed methods.

Methods	PTC taken in synthetic mixture (µg/ml)	% Recovered ± SD[a]
Method-A	20	101.08 ± 0.89
	25	99.80±0.77
	30	100.98 ±0.98
Method-B	0.5	100.22 ± 0.67
	1	99.99±0.98
	1.5	101.98 ±0.99
Method-C	0.5	101.99 ± 0.64
	1	99.79±0.55
	1.5	100.65 ±0.44

[a]Mean value of five determination

Table 6. Results of recovery studies by standard addition method.

Methods	PTC in tablet (µg/ml)	Pure PTC added (µg/ml)	Total PTC found (µg/ml)	Pure PTC recovered [a] Percent ± SD
	10	10	20.01	100.10 ± 1.22
Method-A	10	15	25.18	101.20 ± 1.43
	10	20	30.15	99.00 ± 1.58
	0.25	0.25	0.501	100.40 ± 1.38
Method-B	0.25	0.75	0.995	99.30 ± 1.54
	0.25	1.25	1.51	101.00 ± 1.56
	0.25	0.25	0.499	99.60 ± 1.72
Method-C	0.25	0.75	1.01	101.33± 1.39
	0.25	1.25	1.51	100.00±1.63

[a]Mean values of five determination

Table 7. Results of tablet analysis by the developed methods with reference method.

Tablet brand[a]	Nominal amount (mg)	Reference method[3]	Found % (of nominal amount ± SD)*		
			Developed methods		
			Method-A	Method-B	Method-C
Pivasta	2	99.5± 0.48	101.21±0.56	100.99±0.67	100.96±0.33
			t= 2.15	t=2.34	t=2.11
			F= 1.56	F=1.44	F=1.58
Pivasta	4	98.9±0.85	101.34±0.45	101.30±0.69	101.40±0.99
			t=2.14	t=2.16	t=2.06
			F= 1.34	F=1.48	F=1.08

*Mean value of five determinations

[a]Manufactured by Zydus cadila , India

Tabulated t-value at the 95% confidence level for four degrees of freedom is 2.57

Tabulated F-value at the 95% confidence level for four degrees of freedom is 5.05

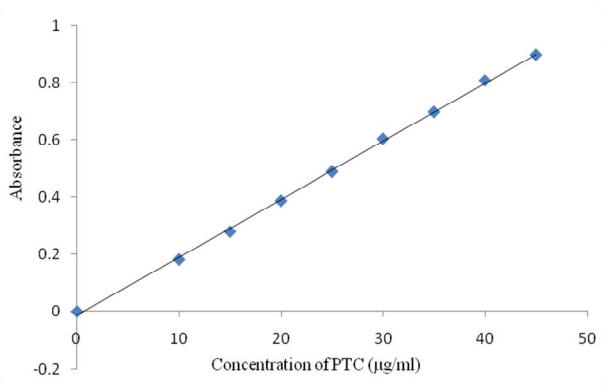


Figure 4. Linearity plot for method A.

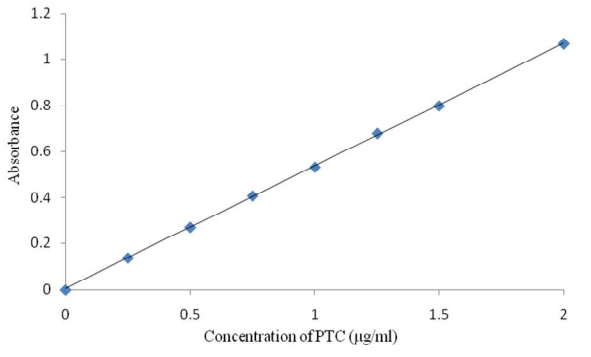


Figure 5. Linearity plot for method B.

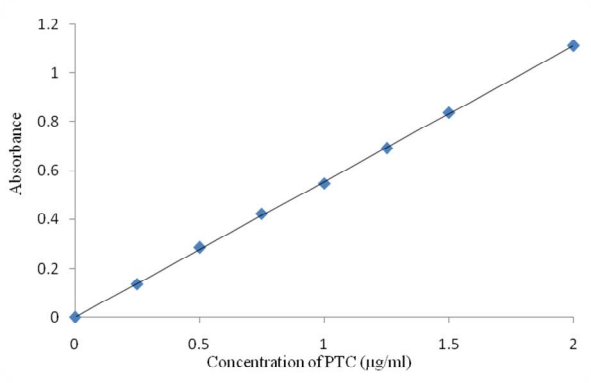


Figure 6. Linearity plot for method C.

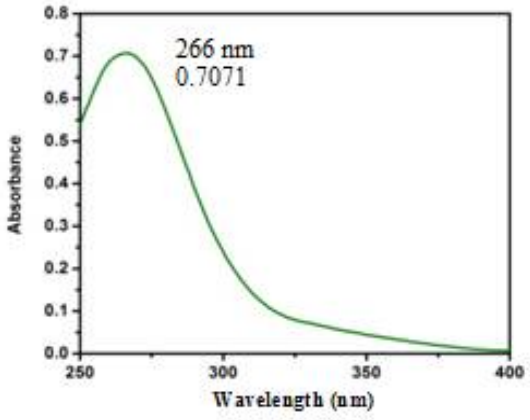


Figure 7. Absorption spectra of HCl reflux at 80°C for 30 minutes.

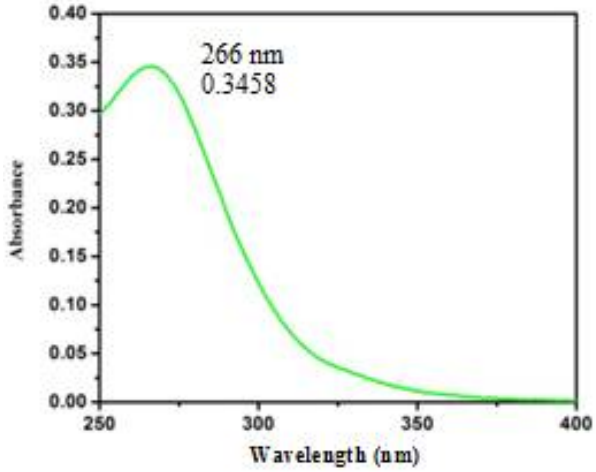
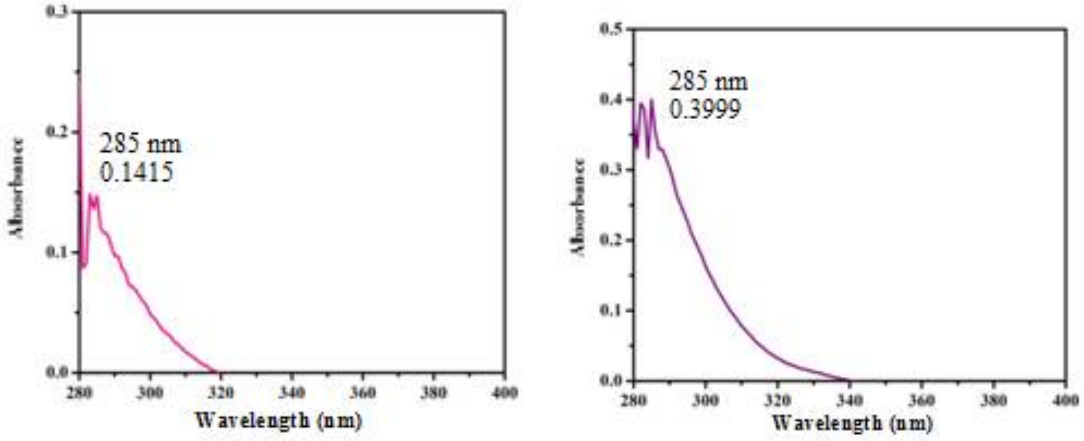
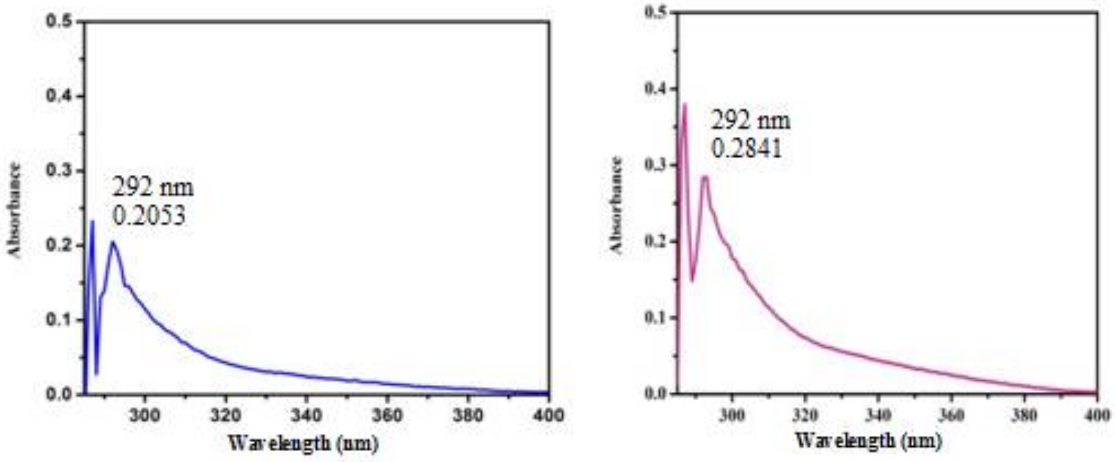


Figure 8. Absorption spectra of NaOH reflux at 80°C for 30 minutes.



(a) (b)

Figure 9. Absorption spectra of (a) 0.5 ml of pure PTC drug + 9 ml of 3% H_2O_2 (b) 1.0 ml of pure PTC drug + 9 ml of 3% H_2O_2.



(a) (b)

Figure 10. Absorption spectra of (a) 0.5 ml of pure PTC drug + 9 ml of 5% H_2O_2 (b) 1.0 ml of pure PTC drug + 9 ml of 5% H_2O_2.

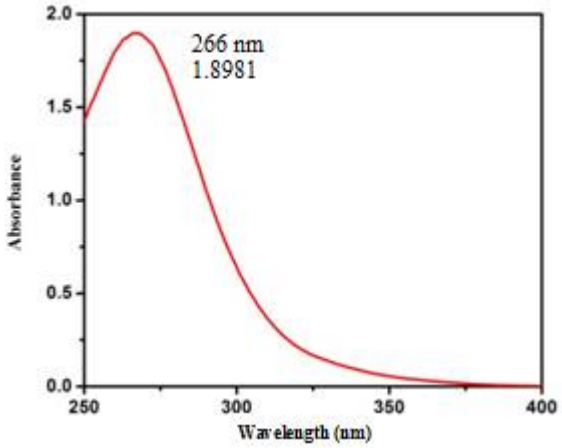


Figure 11. Absorption spectra of thermal degradation of pure PTC at 100ºC.

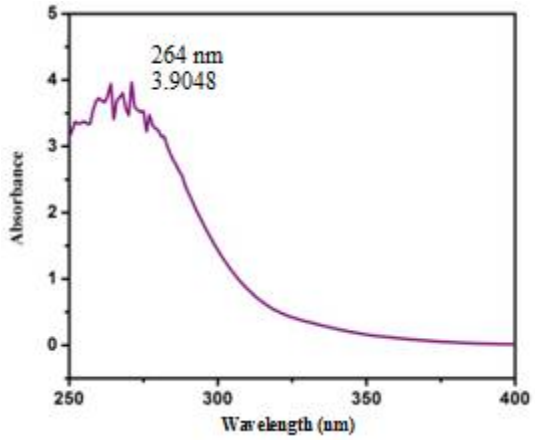


Figure 12. Absorption spectra of UV degradation of pure PTC.

Recovery studies. The accuracy and validity of the developed methods were performed by recovery experiments via standard-addition procedure. The pre-analyzed tablet powder was spiked with pure PTC at three different levels and the total concentration was found by the developed methods. Each determination were repeated for three times and the % recovery was calculated which were in the range of 99.00 to 101.33% with the standard deviation values less than 2%. This result indicated that the percent recovery of pure PTC added within the permissible limits gave the absence of inactive ingredients. The results were illustrated in table 6.

Application to tablet analysis. Commercial PTC tablets were analyzed by the developed methods. The tablet analysis study established that the developed methods were accurate and precise even in the presence of excipients. The obtained results were compared statistically by Student's t-test and students F-test which did not exceed 95% confidence level shown in table 7. This result again indicated that there was a good agreement between the three developed methods and the reference method[3].

Results of force degradation studies. A stress degradation study provides an indication of how the quality of a drug may be affected by the influence of different stress conditions. The degradation products can cause changing of chemical, pharmacological and toxicological properties of drugs having a significant impact on product quality and safety. Pure PTC was found to be quite stable under acid, alkaline and thermal conditions. A slight decomposition was found on the exposure the pure PTC drug to UV degradation as 0.75%. On the other hand, the oxidative 3 and 5% H_2O_2 undergo degradation. The drug decomposed under oxidative degradation was found to be 7.14 and 9.77% indicating that the drug is more sensitive under oxidative condition. Therefore, PTC is more resistant towards oxidative condition when compared to acid, alkaline, thermal and UV degradations.

CONCLUSION

The three developed methods were validated as per ICH guidelines which include the parameters like linearity, precision, accuracy, selectivity and ruggedness. Application of developed methods showed the analysis of PTC in tablet dosage forms where no interference of excipients were found. The developed methods were advantageous over most of the reported methods in terms of sensitivity, simplicity, cost-effectiveness and experimental conditions. The method doesn't involve any tedious procedural steps, any extra reagents or longer analysis time and a very simple instrument is required. The developed methods can be used to determine the purity of pitavastatin calcium from

various sources and also in stability studies. The degradation behaviour of PTC was studied by subjecting PTC under various stress conditions recommended by ICH guidelines. PTC drug undergoes an extensive degradation under oxidative conditions whereas acidic, alkaline, thermal and photolytic stress conditions were quite stable.

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