DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF PITAVASTATIN CALCIUM IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

An isocratic stability-indicating reverse phase high performance liquid chromatographic diode array detection method has been developed and validated for the quantitative determination of pitavastatin calcium in the presence of its degradation products. The chromatographic separation was achieved on Phenomenex Luna C_{18} column (250 X 4.0 mm id, 5µm) in the isocratic mode using acetonitrile-methanol-water (35:25:40, V/V/V, pH 3 adjusted with orthophosphoric acid) as mobile phase. The drug is subjected to different accelerated stress conditions and peaks of the degradation products were well resolved from the pure drug, which indicates the specificity and stability-indicating properties of the method. The method was linear (r= 0.9998) over the concentration range of 5-30 µg/mL. The proposed method was used to investigate the degradation kinetics of PTV in acidic condition at different temperatures. Degradation of pitavastatin followed first-order kinetics, and rate constant (k), half life (t1/2), time left for 90% potency (t90) and energy of activation were calculated.

Keywords: Pitavastatin calcium, Kinetic study, Stability indicating, RP-HPLC-DAD method

INTRODUCTION

Pitavastatin calcium (PTV) [NK-104] is a 3-hydroxy-3-methyl glutaryl coenzyme A reductase inhibitor. It is chemically bis [3r, 5s]-7-[2-cyclopropyl-4-[4-fluorophenyl] quinolin-3-yl] - 3, 5-dihydroxy 6[e] - heptenoic acid calcium salt. Metabolism of pitavastatin by the cytochrome P450 (CYP) system is minimal, principally through CYP 2C9, with little involvement of the CYP 3A4 isoenzyme, potentially reducing the risk of drug-drug interactions between pitavastatin and other drugs known to inhibit CYP enzymes [1-3]. The cellular mechanism of action is attributed to the inhibition of cholesterol biosynthesis in the liver and the drug is the first-line agent for lipid lowering in patients with atherosclerosis and cardiovascular disease[4–7].

The quality of pharmaceutical product of PTV, in terms of purity and stability of the active substance and/or finished product is vital for the effective and safe delivery of its therapeutic values to the patient. This is because the presence of impurities and/or potential degradation products may cause changes of chemical, pharmacological, and/or toxicological properties of the active drug entity[8-10]. In general, pharmaceuticals are sensitive to environmental conditions, including light, temperature, humidity, and the susceptibility of the substance towards hydrolysis or oxidation can play an important role in the production of the impurities/degradation product. These factors usually vary during manufacturing, transportation, storage, and distribution of the finished product. For these reasons, stress testing of the active substance and the finished product is necessary for providing information about the intrinsic stability of drug substances, potential degradation products, possible degradation pathways of the drug, compatibility of the drug with the excipients in the finished product, and the long-term effects of the environmental factors on the active drug and its finished products. Results of stability testing and kinetic determinations are important in developing proper manufacturing process, selecting proper packaging, storage conditions, product's shelf life, and determining the expiration date[11-14].

A literature survey revealed that a different analytical method for analysis of the PTV has been reported. Krishna and Sankar reported spectrophotometric methods based on the oxidation of PTV by ferric chloride in presence of o-phenanthroline (Method A) or 2, 2' bipyridyl (Method B) or potassium ferricyanide (Method C) and colored

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complex formed was measured at 510, 530 and 755 nm for method A, B and C (15). HPLC method for the quantitative determination of PTV in human and dog plasma[16], and in human plasma[17] have been reported. LC-MS/MS method was reported for determination of PTV in human plasma and urine[18-21]. These methods were developed for the purpose of determining the low level of the drug substance in the biological samples, thus they are not suitable for routine analysis of formulation product where the content of API is high in the formulation and require a tedious procedure for sample pre-treatment is required.

High-performance thin-layer chromatography (HPTLC) method has been reported for quantitation of PTV in pharmaceuticals[22-23]. Kumar et al developed a simple HPLC method for determination of PTV in pharmaceutical dosage form[24]. The authors did not study the effect of environmental conditions, including light, temperature, humidity, and the susceptibility of the substance towards hydrolysis or oxidation. Panchal et al developed a simple and stability indicating HPLC method for determination of PTV in pharmaceutical dosage form[25-26]. They used triethylamine as a component of the mobile phase. Longer saturation time is needed when amino derivative (diethylamine or triethylamine) modifier is used as a component of mobile phase, which is not recommended for routine analysis of the drug content in the pharmaceutical industry. It also causes bonus troubles during analysis, such as erratic baselines and poor peak shape[29]. Pawel et al developed an LC method to study photostability of pitavastatin using phosphate buffer as a mobile phase component[27]. Buffer can damage the column, which can make the procedure less attractive for routine analysis[29]. Stability indicating UPLC method for determination of pitavastatin has been reported[28]. Thus, above methods cannot be useful for routine analysis due to tedious mobile phase composition such as phosphate buffer and problems arising from triethylamine.

However, there has been no report of a precise kinetic study concerning the degradation of PTV in acidic condition. Moreover, kinetic studies and accelerated stability experiments are important to solve problems encountered in quality control and to predict the expiry dates of pharmaceutical products. The scientific novelty of the present work is that the suggested method represents the first kinetic study of PTV degradation using RP-HPLC-DAD. In addition, it is more efficient and accurate than the reported HPLC methods. Present study involves the development and validation of stability indicating and sensitive LC method with simple mobile phase for quantitative determination of PTV in pharmaceuticals without using triethylamine and buffer content in the mobile phase. The kinetic parameters, such as the apparent order degradation rate constant (k), half-life ($t_{1/2}$), and energy of activation for PTV were calculated. Determination of the shelf life, t_{90} for the PTV in acidic conditions, assuming that the product was satisfactory until at the time at which it has decomposed to 90% of its original concentration (i.e. 10% decomposition) at different temperatures, was carried out.

EXPERIMENTAL

Chemicals, Reagents and Standards

Pitavastatin calcium was obtained from Cadilla Pharmaceuticals Ltd, Ahmedabad, India. HPLC grade acetonitrile, methanol and water were procured from Merck (Mumbai, India). Hydrogen peroxide (Chemdyes Corp., Ahmedabad, India) and ortho phosphoric acid (AR grade, S.D.Fine Chemicals, Ahmedabad, India) were of analytical grade used. PTV Pitava® tablets (Zydus Pharmaceutical Ltd., Ahmedabad) containing 2mg PTV/ tablet were evaluated.

Instrumentation

The development and validation of the LC method was performed on a Shimadzu (Kyoto, Japan) liquid chromatography equipped with an LC-2010AHT, SPD-M10A photo diode array (PDA) detector, auto sampler and Class-vp chromatography software**.** The detector was set at 244 nm**.** The analytical column was Phenomenex (Torrence, CA) Luna C_{18} column (250 x 4.6 mm id; 5µm particle size). The column temperature was kept constant at 25 ± 2 °C. Digital pH meter (Eutech Instruments, Model: pH tester-1). Shimadzu electronic balance model AUX-220 and ultrasonic cleaner (Frontline F_{54}, India**)** were used.

Chromatographic Conditions

The chromatographic separation was performed in isocratic mode using reverse phase C_{18} column equilibrated with mobile phase acetonitrile, methanol and water (35:25:40, V/V/V), and apparent pH adjusted to 3.0 with orthophosphoric acid. The flow rate of the mobile phase was 1.5 mL/min and the sample injection volume was 20 µL.

Preparation of standard solution

An accurately weighed quantity (100 mg) of the PTV standard drug was transferred to a 100mL volumetric flask, and dissolved in and diluted to the mark with methanol to obtain the standard stock solution of 1 mg/ mL of PTV. This stock solution was further diluted with mobile phase to obtain a working standard solution of 100µg/mL PTV.

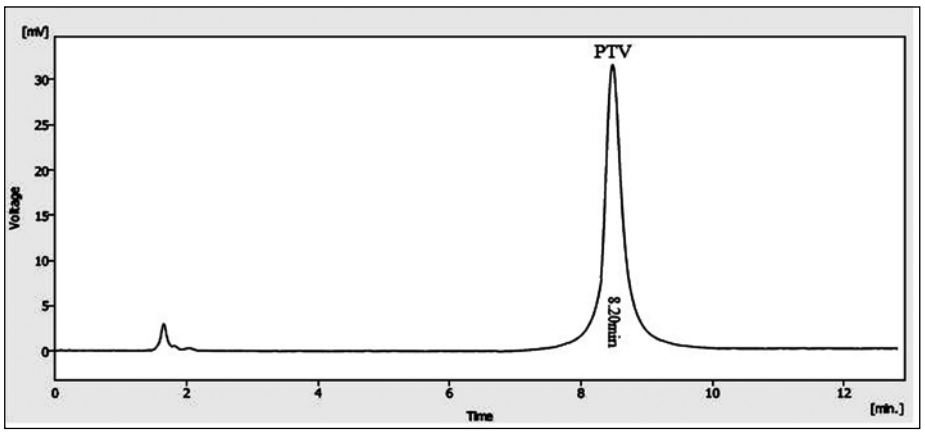

Figure 1 Liquid Chromatogram of Pitavastatin calcium at 244nm

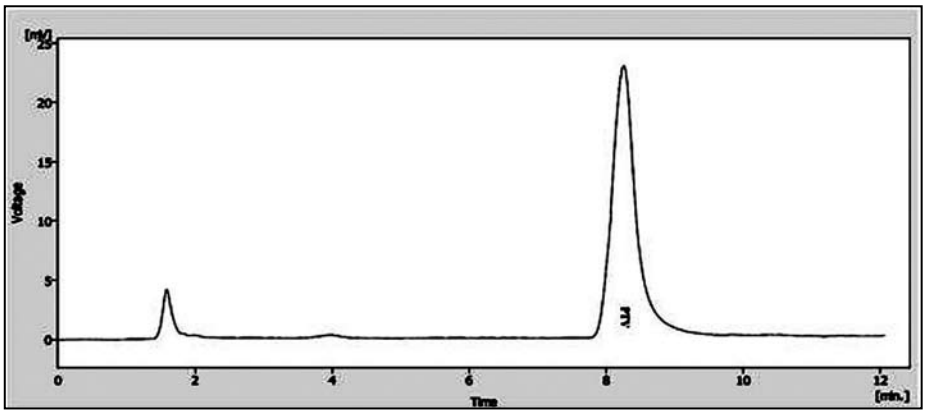

Figure 2 Chromatogram of base (0.1M NaOH) treated pitavastatin at 80°C after 120min

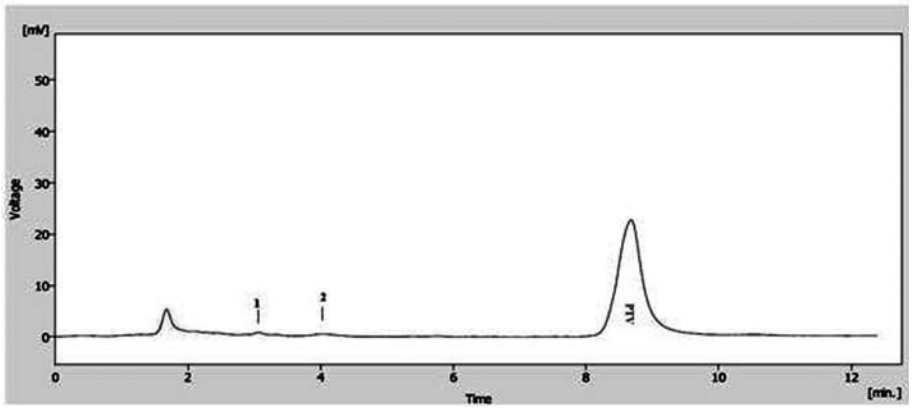

Fig. 3: Chromatogram of hydrogen peroxide treated pitavastatin at 80°C after 120min

Stress studies

The samples for degradation studies were prepared by transferring 5 mL solution of PTV (1mg/mL) to three different 50 mL volumetric flasks. Sodium hydroxide solution (5 mL, 0.1M), hydrochloric acid solution (5 ml, 0.1M) and hydrogen peroxide solution (5 mL, 3%) were added in separate flasks for alkaline hydrolysis, acidic hydrolysis and oxidative degradation, respectively. The solutions were heated at 80°C for 120 min in thermostatically controlled oven, cooled to room temperature, neutralized (for acid and base induced degradation), appropriately diluted with mobile phase to obtain a final concentration of 10 µg/mL of PTV and injected in to the chromatographic system.

Thermal degradation was attempted by heating powdered drug (in a 1 mm thick layer in a petri plate) at 80°C in oven for 24 h. Photo degradation was attempted by exposing powdered drug (in a 1 mm thick layer in a Petri plate) to UV light (λ =254 nm) in the photo stability chamber for 24h. After the specified time, the samples were cooled to room temperature. 10 mg of PTV was weighed and dissolved in methanol. The solution was appropriately diluted with mobile phase to obtain a final concentration of 10 µg/mL of PTV and injected in to the chromatographic system. The degraded samples were analyzed by comparison with a control sample (lacking degradation treatment). The forced degradation studies were carried out in the dark to exclude the possible degradation effect of light.

Kinetic study

The kinetic acid degradation of PTV was evaluated in 0.1 M HCl at 80°C, 70°C, 60°C and 50°C for different time periods. Solutions containing 1 mg/mL of the PTV were prepared in methanol. Appropriate aliquots of these solutions were transferred into separate stoppered volumetric flasks, and diluted with 0.1 M HCl to give a final concentration of 100µg/mL PTV. The flasks were placed in a thermostatic oven at different temperatures (80°C, 70°C, 60°C, 50°C) for different time intervals of 30 min, 60 min and 120 min. Three samples were analyzed for each time interval. After the specified time,

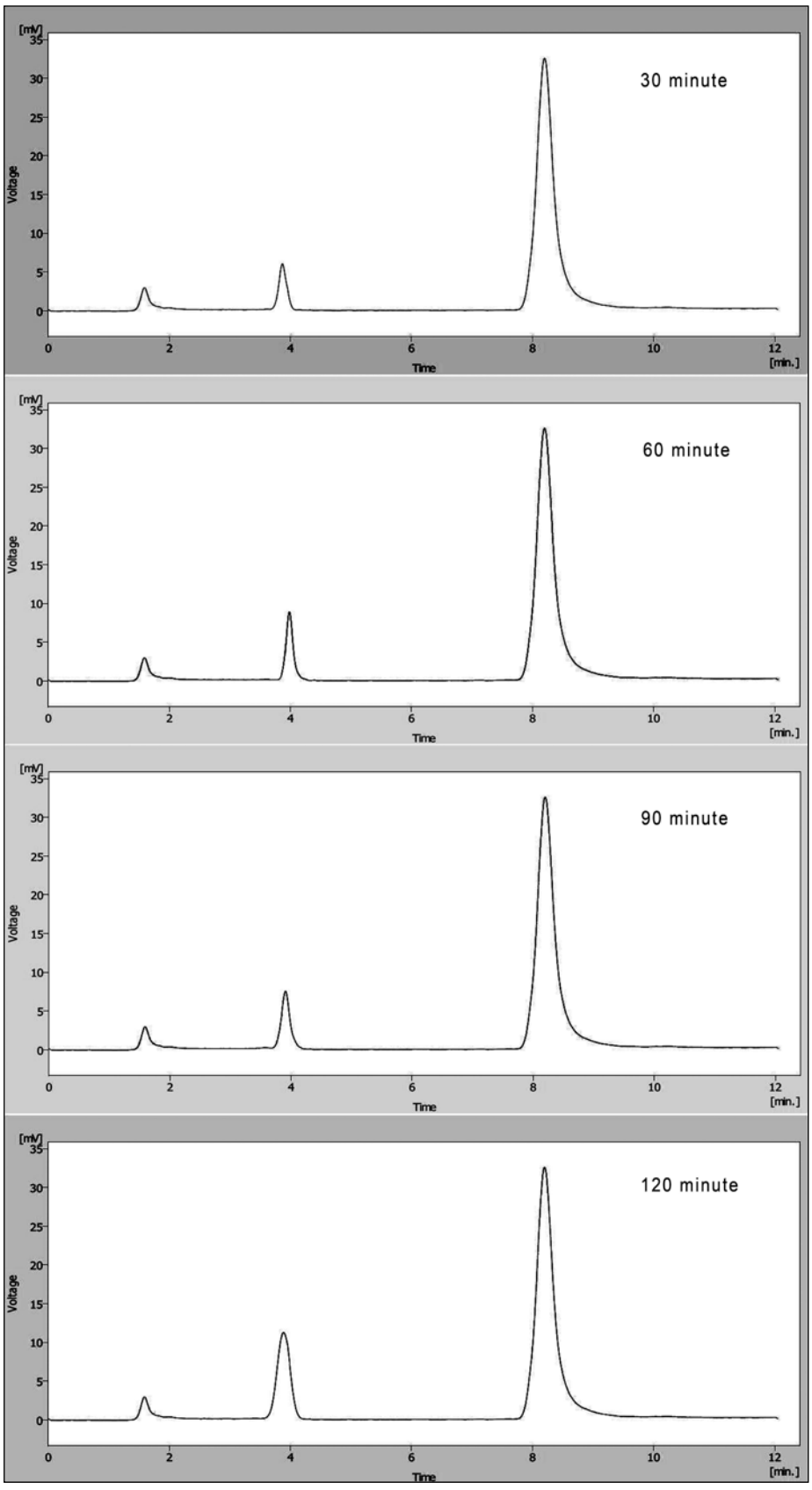

Fig. 4: Chromatogram of acid (0.1M HCl) treated pitavastatin at 80°C after 30min, 60min, 90min and 120min

1 mL aliquots were transferred to separate 10 mL volumetric flasks and neutralized with 0.1 M NaOH was checked by using pH strip and these solutions were diluted with mobile phase. These solutions were injected in the HPLC system and the concentration of the intact PTV were calculated of each sample.

The concentrations of the intact PTV determined at the different time intervals, were used in the kinetic determination plots. The kinetic parameters, such as the apparent order degradation rate constant (*k*), half-life ($t_{1/2}$), t_{90} and energy of activation were calculated.

Analytical Method Validation

The methods were validated according to the United States Pharmacopeia, 31st edition (2008) and ICH Guidance for Industry (International Conference on Harmonization 2005).

Specificity

The specificity of the method was ascertained by analyzing PTV from standard and sample solution of the tablet dosage form or PTV from stressed samples. The identity of the peak in the sample and stress sample was confirmed by comparison of the retention time and UV spectrum of the peak from the sample and stressed sample with those of the peak from the standard. Peak purity for the PTV peak was assessed by comparing UV spectra acquired at the peak start (S), peak apex (M) and peak end (E). The specificity of the proposed HPLC method was proven by its ability to determine PTV in commercial tablets and stressed sample, confirming that there was no interference by common excipients, additives or forced degradation product of PTV.

Table I: Summary of stress study of pitavastatin calcium at 80°C

Condition	Time	Recovery (%)	Retention time of degradation product
Acid stress sample (0.1M HCl)	2 hr	76.16 %	3.97 min
Base stress sample (0.1M NaOH)	2 hr	96.5 %	4.05 min
Peroxide stress sample (3% Hydrogen Peroxide)	2 hr	95.52 %	3.2 min, 4.05 min
Thermal stress sample (Heated at 80°C)	24 hr	96.45%	-
Photo light stress sample	24 hr	98.3%	-

Table II: Kinetic parameters of pitavastatin calcium in acidic condition (0.1M HCl)

Temperature (°C)	K_{obs}(min^{-1})	$t_{1/2}$ (min)	t_{90} (min)
60	5.96 X 10^{-3}	1163	176
70	8.69 X 10^{-3}	797	121
80	1.69 X 10^{-2}	410	62
90	2.19 X 10^{-2}	316	48

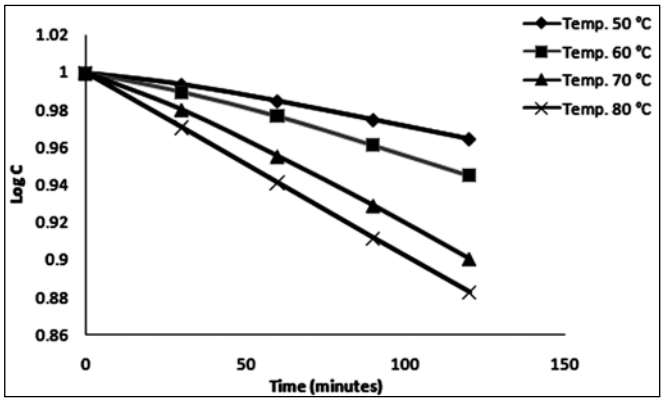

Fig. 5: First order plot of the pitavastatin in 0.1M hydrochloric acid at different temperature by LC method

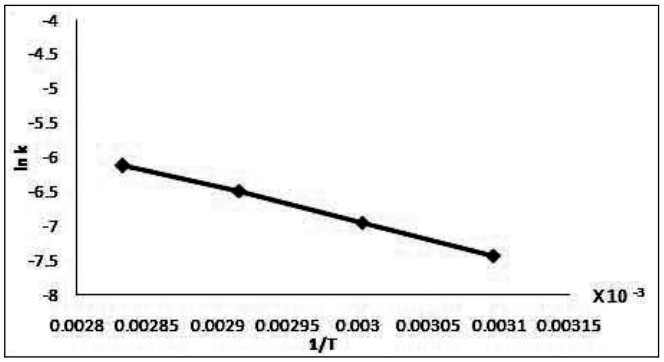

Fig. 6: Arrhenius plots for the degradation of pitavastatin in 0.1M hydrochloric acid by LC method. K is apparent first order degradation rate constant and T is temperature on Kelvin scale.

Table III: Summary of validation parameter

Parameter	Pitavastatin calcium
Linearity and range	5-30 µg/mL
Correlation coefficient (r)	0.998
DL [a]	0.63 µg
QL [b]	1.91 µg
Accuracy (% Recovery)	99.08- 101.21%
Precision (% RSD[c]) Inter day (n=3) [d] Intraday (n=3) [d]	 0.478-0.714 0.399-0.576
Robustness	Robust
Specificity	Specific

[a] *DL = Detection Limit*

[b] *QL = Quantitation Limit*

[c] *RSD = Relative Standard Deviation*

[d] *n= Average of three determination a*t each

Linearity and Range

To study linearity and range, the concentration range 5 µg/mL - 30 µg/mL samples in prepared by pipetting out appropriate aliquots of working standard solution (100µg/mL) in different 10 mL volumetric flasks and resultant solution was diluted up to 10mL with the mobile phase to obtain a final concentration of 5, 10, 15, 20, 25 and 30 µg/mL of PTV.

Precision

Method precision was evaluated as repeatability (or intraday precision) and intermediate precision (interday precision), in accordance with ICH guidelines. Repeatability (intraday precision) was performed at three different concentrations 10, 20 and 30 µg/mL of PTV by estimating the corresponding responses three

Table IV: Robustness study

Variables	Optimized Condition	Modify in condition	% RSD (Peak Area)
Flow Rate	1.5 mL/min	1.4 mL/min	0.55
		1.6 mL/min	0.61
pH	3	2.8	0.57
		3.2	0.73
Mobile phase ratio	Acetonitrile-methanol-water (35:25:40, V/V/V),	30+ 25 +45	0.95
		35+ 20 +45	0.37
		40+ 25+35	0.53
Column oven temperature	25 ^{0}C	23 ^{0}C	0.19
		27 ^{0}C	0.24
UV detection wavelength	244 nm	242 nm	0.58
		246 nm	0.69

Table V: System suitability test parameters

System Suitability Test Parameters	PTV	% RSD
Retention Time (R_t)	8.20	0.04
Theoretical Plate number (N)	6856	0.98
Tailing Factor (A_s)	1.12	0.18

times on the same day and under the same experimental condition. The results were reported in terms of % RSD. The intermediate precision (interday precision) was performed at the concentration of 10, 20 and 30 µg/mL PTV on three consecutive days. The results an reported in terms of % RSD.

Accuracy

Accuracy was assessed by determining the agreement between the measured analyte concentrations of the fortified and unfortified sample and the known amount of analyte added to fortify the sample. An amount of standard and an amount of powder of tablets equivalent to 10 mg were separately and exactly weighed and transferred separately to 100 mL volumetric flasks. Standard and sample solutions were prepared separately as described to obtain solutions containing 100 µg/mL of PTV. Final concentrations of 18, 20 and 22 µg/mL PTV solutions were prepared and analyzed. Three replicate samples of each concentration level were prepared and the percentage recovery at each level (n=3) was determined.

Robustness

The methods robustness was assessed by small but deliberate changes made in the composition of the mobile phase, flow rate, pH, UV detection wavelength and column oven temperature. The standard solution containing PTV was injected three times by changing the following parameters and % RSD of peak area were calculated.

a) Change in composition organic phase of mobile phase is following:

Acetonitrile:Methanol:Water (30: 25: 45)

Acetonitrile: Methanol: Water (35: 20: 45)

Acetonitrile:Methanol:Water (40: 25: 35)

b) Change in pH of mobile phase is 2.8 and 3.2.

c) Change in the flow rate of the mobile phase is 1.4mL/min and 1.6mL/min.

d) Change in detection wavelength is 242nm and 246nm.

e) Change in column temperature is 23 ^{0}C and 27 ^{0}C.

Detection Limit (DL) and Quantitation Limit (QL)

Sensitivity of the method was determined with respect to detection limit (DL) and quantitation limit (QL). DL and QL were calculated using following equation as per ICH guidelines.

$DL = 3.3´ s/S$

$QL = 10´ s/S$

where s is the standard deviation of response and S is the slope of the calibration curve.

System suitability test

A system suitability test was used to verify that the resolution and repeatability of the system were adequate for the analysis intended. The system suitability parameters such as, retention time, asymmetry of the chromatographic peak (Tailing factor) and number of theoretical plate (N) were evaluated for six replicate injections of PTV and results are reported in terms of %RSD.

Analysis of tablet formulation

Twenty tablets were weighed accurately and crushed to a fine powder. A quantity of powdered tablet equivalent to 25 mg of PTV was transferred in to 25mL of volumetric flask and 10mL of methanol was added. The solution was sonicated for 15 min and diluted up to mark with methanol. This solution was filtered through 0.45µm nylon membrane filter. This solution was further diluted with the mobile

phase to obtain a working sample solution of 10µg/mL PTV and analyzed by the proposed method.

RESULTS

Method Development and Optimization

The mobile phases were composed of acetonitrile-methanol-water (35:25:40, V/V/V) with pH adjusted to 3 with orthophosphoric acid. The retention time of PTV was found to be 8.20 ± 0.05min (Fig. 1). The proposed method is simple and without complex mobile phase (i.e., phosphate buffer, triethylamine) in comparison with the published methods described in the literature and can be used by quality control laboratory.

Stress studies

The acid induced degradation study, pitavastatin calcium showed decrease in PTV peak and showed one additional peak at retention time 3.97min (about 23.84% degradation) Fig. 4 (a), (b), (c), (d)). In the base induced degradation study, pitavastatin calcium showed decrease in PTV peak and very less prominent additional peak at retention time 4.05min (about 3.5% degradation) (Fig. 2).

The *oxidative* degradation study, of pitavastatin calcium showed decrease in PTV peak and two additional peaks at retention time 3.2 min and 4.05 min (Fig. 3). Upon dry heating of pitavastatin calcium, a decrease in the original drug peak area was observed, and no additional peaks were observed in the chromatogram. Pitavastatin calcium is exposed to light in photo stability chamber, 1.7% degradation is observed and no additional peaks were observed in the chromatogram. Summary of stress condition and results are shown in the Table I. The stress study was performed in the dark to exclude the possible degradation effect of light.

Kinetic study

The chromatograms obtained revealed that the peak area of PTV was reduced with time. It was found that the major degradation product (P1) of PTV was in acidic condition at retention time (Rt) 3.97 ± 0.03(Fig. 4 (a), (b), (c), (d)). At the selected temperature (50, 60, 70 80°C) the acid degradation of PTV processes first-order kinetics (Fig. 5). Plotting log K values versus 1/T, the Arrhenius plots (Figure 6) were obtained, which were found to be linear in the temperature range 50–80°C for the acidic degradation (r= 0.977). The activation energy was calculated for PTV which was found to be 10.364kcal/mol. From the slopes of the straight lines, it was possible to calculate the apparent first order degradation rate constant, the half life and time left for 90% potency (t_{90}) at each temperature a shown in Table II.

METHOD VALIDATION

The peak purity indices of the analyte in stressed solutions were found to be greater than 0.9997, indicating that no additional peaks were co-eluting with each of the analyte.

PTV showed linearity over the concentration range of 5-30 µg/mL. Peak area and concentration were subjected to linear least-squares regression analysis to calculate the calibration equation and correlation coefficient (r^2= 0.996 ± 0.002). The regression equation obtained was:

y = 78142 x + 30737,

where *y* = Peak area and *x* = concentration of PTV (µg/mL). The results of intra-day and inter-day precision studies an shown in Table III.

Accuracy study was performed by recoveries of the added analyte to standard from calibration curves. The recovery found was in the range of 99.08% ± 0.89 - 101.21% ± 0.84, % RSD value was 0.794 - 1.164. Robustness study results are shown in Table IV. The % RSD value was found to be less than 2, indicating the robustness of the method. The DL and QL for PTV were found to be 0.63 and 1.91 µg/mL indicating the sensitivity of the proposed method. Summary of validation parameters are shown in Table III.

System suitability

The % RSD values calculated in the system suitability test for the parameters studied were within the acceptable range (% RSD < 2.0%), as shown in Table V, indicating that system is suitable for the analysis intended.

Method application

The proposed RP-HPLC-DAD method was applied to the determination of PTV in tablet dosage form without prior separation of the excipients of the formulation. The results obtained were comparable with the corresponding label claim percentages 103.76 % ± 1.96 (S.D.) demonstrate the quality of the analyzed pharmaceutical samples and the applicability of the method for QC analysis.

DISCUSSION

Method development and optimization

The main criteria is to develop successful HPLC method for determination of PTV and its degradation product, the method should be able to determine assay and degradation product of drugs in a single run.

Selection of Mobile phase composition

To develop efficient and reliable stability-indicating RP-HPLC-DAD method for quantitative determination of PTV in a short time of analysis, different mobile phases were tested to achieve efficient separation of degradation products formed under stressed conditions and excipients. Several strategies have been tested to achieve a capacity factor (k) >1 and to provide sufficient selectivity and sensitivity short analysis time. The objective of this research was to obtain rapid and economical chromatographic separations with good peak symmetry using simple mobile phases without the presence of triethylamine or buffers, which can damage the column in routine analyses [29]. On the other hand, complex mobile phases require specific reagents.

In order to separate PTV and degradation products produced under stress conditions, different mobile phases were used and adjusted to obtain a rapid and simple assay method with a reasonable run time, suitable retention time, and good peak symmetry. Optimization of mobile phase was performed based on the resolution of the drug and its degradation products, asymmetric factor and theoretical plates obtained for PTV. Different ratios of methanol, water and acetonitrile were evaluated as a component of the mobile phase. Acetonitrile was chosen because it improves the peak symmetry and needs short analysis time. The use of methanol resulted in better sensitivity (0.63µg/mL). The drug was eluted with a diluents peak, which was splitting in nature. When pH was equal to pKa values (PTV pKa = 4.0), more splitting was observed. The retention times of statins are extremely pH sensitive, especially when pH of the mobile phase is more than 3.2 or less than 3.0. When the pH of the mobile phase was adjusted to 3.0 with O-phosphoric acid, the retention time was around 8 min; PTV and degradation products were separated and the symmetry of the PTV peak was improved. The resolution among all degradation products was also improved. Therefore, final mobile phases were composed of acetonitrile-methanol-water (35:25:40, V/V/V) with pH adjusted to 3 with orthophosphoric acid and the retention time of PTV was found to be 8.20 ± 0.05min (Fig. 2). UV spectra of PTV standard solution scan between 200-400nm showed that the drug PTV absorbed considerably at 244 nm, so the same was selected as the detection wavelength.

Stress study and kinetic study

Forced degradation or stress testing is undertaken to demonstrate specificity when developing stability-indicating methods, particularly when little information is available about potential degradation products. The ICH guideline entitled “Stability Testing of New Drug Substances and Products” requires stress testing to be carried out to elucidate the inherent stability characteristics of the active substances[14]. The degradation of most pharmaceuticals can be classified as zero order, first order, or pseudo first order[30]. Thus, kinetic studies of the decomposition of drugs using stability testing techniques are essential for the QC of such products to predict the expiration date of drug. Among the tested conditions, degradation was mild in alkaline hydrolysis, oxidative, photolysis and thermal conditions compared to acid hydrolysis. In all the above cases, the degradant peaks did not interfere with the PTV peak, suggesting that the method enabled specific analysis of PTV in the presence of its degradation products.

The kinetic study of PTV was performed in 0.1MHCl at different temperature (50, 60, 70 80°C). It was found that the P1 was major degradation product of PTV in acidic condition at retention time (Rt) 3.97min, which does not interfere with the detection of PTV (Rt=8.20min). The kinetics parameters are shown in Table II. Rate constant, time left for 50% of potency ($t_{½}$), time left for 90% potency (t_{90}) or 10% decomposition of PTV in acidic condition were calculated using equations (1), (2) and (3), respectively[30].

$$k = \frac{2.303(\text{Log}C_o - \text{Log}C_t)}{t} \qquad \text{.....(1)}$$

$$t_{½} = \frac{0.693}{k} \qquad \text{.....(2)}$$

$$t_{90} = \frac{0.105}{K} \qquad \text{.....(3)}$$

The influence of temperature on the reaction rate constant (k_{obs}) in 0.1MHCl was given by the Arrhenius equation (4)[30].

$$\ln k_{obs} = \ln A - \frac{E_a}{RT} \qquad \text{.....(4)}$$

where A is the frequency factor, E the energy of activation, R the universal gas constant, and T is the absolute temperature.

Method Validation

The peak purity indices of the analyte in sample and in stressed solutions, determined with a PDA detector under the optimized chromatographic conditions, were found to be greater than 0.9997, indicating that no additional peaks were co-eluting with each of the analyte and evidencing the ability of the method to assess unequivocally the analyte of interest in the presence of degradation product. Peak purity index indicated that the proposed method is specific and stability-indicating, and can be applied for stability studies and QC analysis of PTV in pharmaceutical products and stressed samples.

The developed method was found to be precise on the basis of the low RSD values for the repeatability and intermediate precision studies, which were <2 %.

Accuracy was evaluated by the simultaneous determination of the analyte in solutions prepared by the standard addition method. Known amount of the standard at 80%, 100% and 120% levels was fortified to the degradation sample. The results (Table III) revealed low bias and essentially quantitative recoveries, indicating that the method enables the accurate determination of the analyte.

The effects of deliberate variations in the pH, composition of the mobile phase, the flow rate, UV detection and column oven temperature on instrumental response (peak area) were evaluated. There were no significant difference changes in the results when the modifications were made in the experimental conditions, indicating that the proposed method was robust.

CONCLUSION

The first stability indicating RP-HPLC-DAD method was developed and validated; it was simple, fast, precise, and accurate without having to use triethylamine and complex buffer in the mobile phase. The results of stress testing were undertaken according to the ICH guidelines, which revealed that the method is specific and stability-indicating. Based on the peak purity results, the method is specific for the determination of PTV in the presence of degradation products. A kinetic study of PTV was evaluated in acid medium at different temperature by RP-HPLC-DAD method. The acidic degradation of PTV followed a first order reaction. It may be extended to determination of the degradation kinetics of PTV in biological fluids. The proposed method was successfully applied to determine the PTV content in tablet dosage form within acceptable limits and may be applied for assay, dissolution studies, bio-equivalence studies, as well as to accelerated stability studies to predict the expiry dates of pharmaceuticals.

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