Development And Validation Of New Stability Indicating Method For Assay Of Pitavastatin In Solid Oral Dosage Form.

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Abstract:
To explore recent advancements in HPLC instrumentation and column technology for stability-indicating analysis. Pitavastatin (PVS) is a synthetic competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and a very effective lipid-lowering medication. Aim of this study was to develop, optimize and validate novel stability-indicating RP-HPLC methods for Pitavastatin in solid oral dosage forms.

Keywords: Pitavastatin, RP-HPLC, Analytical techniques, Validation, stability-indicating analysis

Introduction:
Pitavastatin (PVS) is a synthetic competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and a very effective lipid-lowering medication. Pitavastatin pills' initial and ongoing dosing should be tailored to each patient's specific needs, taking into account factors including treatment objective and response. [1]
The analytical techniques used to check the quality of active pharmaceutical components and medicinal products are expanding to include more functionalities as validation develops. The term "validation" is used to describe a specific procedure in quality assurance, of which there are several possible variants. As a result, there is no one set procedure for ensuring accuracy. This research work was aimed to develop stability-indicating RP-HPLC methods for specific pharmaceuticals in solid oral dosage forms. [2]

Materials and method
Pitavastatin tablets were procured from Kowa Pharmaceuticals America, Inc, Sun Pharmaceuticals, Lupin, Mylan and Pitavastatin API from Mylan Laboratories Limited. Potassium Dihydrogen phosphate, Sodium Hydroxide, Orthophosphoric acid, Acetonitrile, Hydrochloric acid, Water was procured from Merck.

Method development and optimization
The steps of sample pretreatment, chromatographic separation, detection, and quantification must all be evaluated and optimized before a final method can be developed. A selective and sensitive approach for HPLC analysis employing UV detection was developed by optimizing a number of parameters.

Buffer preparation
Dissolved 7.0 g of potassium dihydrogen phosphate in HPLC water and diluted it to 1000 ml in a 1000 ml beaker for accurate weighing. Used orthophosphoric acid to get the pH level down to 3. Used a 0.45 m membrane filter to purify the solution.

Preparation of mobile phase
Prepared a suitable quantity of mixture of buffer and acetonitrile in the ratio of 35:65 (v/v). Mixed and degassed.

Chromatographic conditions

Column	Symmetry C18 (Xttera); 150 x 4.6 mm; 5µm
Column temperature	30°C
Flow rate	0.7 mL / minute
Injection volume	30 µL
Detector Wavelength	345 nm
Run Time	10.0 minutes
Pump mode	Isocratic

Preparation of standard stock solution
Pitavastatin working standard, about 100 mg, was weighed and transferred to a 100 mL volumetric flask. To dissolve the contents, 70 mL of diluent was added and sonicated. Added a diluent to bring the volume up to specification and stirred well. We've taken 4.0 ml of this solution and diluted it to 100 ml using diluents.

Test Sample preparation
Not fewer than 30 4 mg pills were carefully weighed, and their respective masses were recorded. After that, we determined the median mass. Used a mortar and pestle to reduce the pills to a powder. Put the amount of Pitavastatin powder in a 100 mL volumetric flask that corresponds to 100 mg after careful weighing. Sonicated at high speed for 30 minutes with 70 mL of diluent, then let it cool to room temperature. Dilution brought the volume up to specification, and the mixture was smooth. Permitted the excipients to reach equilibrium. To make 100 ml, dilute 4 ml of the aforesaid solution with the diluent. Used a PVDF filter with a 0.45 m pore size to purify the solution.

Optimization of experimental conditions
The chromatographic conditions were optimized by factoring in 1) the column that was used Thirdly, mobile phase composition Thirdly, the pH of the transport medium 4) The mobile phase flow rate 5), the range of the injected particles, and 6), the detecting wavelength.
Resolution, asymmetry factor, and peak area data for Pitavastatin guided the optimization of the mobile phase. Symmetric peaks of Pitavastatin were obtained using the mobile phase buffer pH 3.0 and acetonitrile with isocratic elution, which was considered to be adequate. The 150 × 4.6 mm dimensions of the column were deemed preferable than the 100 x 4.6 mm ones. The flow rate was tuned by balancing the run duration and system parameters at 0.7 ml/min. The highest molar absorptivities of the medication determined that 345 nm was the optimal wavelength. In order to produce a symmetric peak and avoid shifting of peak retention time, the column oven temperature was tuned at 30 °C, which results in a shorter run time but more than the limit of tailing. The peak became more diffuse as the sample volume increased, whereas the peak form was optimal for an injection volume of 30 L. Experiments were run in series, with each injector used once, to fine-tune the system's settings. [3]

Method validation
Analytical solution validation of the HPLC technique demonstrated its specificity, linearity, precision, accuracy, ruggedness, and stability. The established procedure was validated in accordance with ICH and FDA guidelines. [4]

Specificity
Placebo interference
The impact of placebo was investigated in a recent research. Preparation of samples for HPLC analysis included injecting a weighted equivalent of a placebo solution into the apparatus, as specified by the test method. In Fig. 3.1 of the Chromatogram, we can see that the peak is uniform, that the excipients do not interfere with the retention time of the analyte peak, and that there are no co-eluting peaks.

Interference from degradation products
To prove the stability and specificity, a forced degradation study was performed. To hasten their breakdown, we subjected placebo and medication samples to the conditions listed below.
Treated with 1N HCl solution.
Treated with 5N NaOH solution.
Treated with 30% Hydrogen peroxide.
Exposed to Humidity at 35°C, 90% RH for about 73 hours.
Exposed to Heat at about 105°C temperature for about 73 hours.
Exposed to UV light for about 73 hours.

The test procedure dictated the analysis of the stressed samples. Pureness of the peak was determined by analyzing the chromatograms of the stressed samples. The peak purity of all the forced degradation samples was determined to be well within the allowed range. Peak homogeneity and the absence of co-eluting peaks, as demonstrated, suggest the stability indicating the distinctive nature of the approach. (Acceptance criterion: %Degradation no more than 30, Purity angle no greater than Purity threshold.

Mode of degradation	Conditions		Pitavastatin		
		Assay (mg/ Tab)	% Degradation w.r.t. control	Purity Angle	Purity Threshold
Control	No treatment	4.08	-	1.348	3.638
Acid degradation Conc., 1N HCl	Heated on boiling water bath for 15 min.	3.68	9.8	3.647	3.931
Alkali degradation 5N NaOH	Heated on boiling water bath for 180 min.	3.83	6.37	3.687	3.619
Peroxide degradation 30% W/V H_3O_3	Heated on boiling water bath for 180 min.	3.74	8.33	3.399	3.318
Thermal degradation	105°C/3days	3.41	16.43	3.317	3.391
Photolytic degradation	UV light stressed (300 Watts hours per Sq. m) 3days	3.87	5.15	3.553	3.614
Humidity degradation	(35°C/90%RH-3 Days)	3.91	4.17	3.618	3.386

Linearity

By constructing a graph of concentration vs average area counts of the analytes, we were able to verify the linearity of the detector's response. The method M1's response is linear across the given range. The correlation coefficient must be at least 0.999 to be considered acceptable.

% Level	PITAVASTATIN	
	Concentration (µg/mL)	Average area counts
LOQ	0.0088	1348
70	38.36	3991503
80	33.31	4569334
90	36.33	5133458
100	40.38	5701939
110	44.43	6349873
130	48.46	6793481
130	53.51	7393483
Slope(b)		140340.5415
Intercept(a)		41166.4459
Correlation Coefficient(r)		0.9995

Precision

System precision The HPLC system received five independent injections of the reference solution. The analytical system meets the accuracy requirements (Acceptance criterion: RSD should not be higher than 1%),.

Injection No.	Area counts
1	5687178
3	5685195
3	5707431
4	5706065
5	5690380
Mean	5695330
SD	10680.6
RSD (%)	0.19

Method precision

By preparing and testing six samples from the same batch, we were able to assess the accuracy of the suggested procedure.

Samples No.	Assay (mg/tab)
1	4.05
3	3.95
3	3.97
4	4.06
5	3.99
6	4.05
Mean	4.01
SD	0.05
RSD (%)	1.18

Ruggedness

Precision testing was performed by employing several HPLCs and columns of the same brand and by different analyzers on separate days to confirm the method's robustness. Six samples were made and analyzed using the suggested approach, all from the same batch. The total RSD difference between the two data sets is a good measure of how stable the approach is. Overall RSD must be less than or equal to 3% to meet the acceptance requirement. [5]

Samples No	Assay (mg/tab)	
	Set-I	Set-II
1	4.05	3.99
3	3.95	4.08
3	3.97	3.96
4	4.06	4.03
5	3.99	3.89
6	4.05	3.96
Mean	4.01	3.99
SD	0.05	0.06
RSD (%)	1.18	1.61
Overall Mean	4	
Overall SD	0.05	
Overall RSD (%)	1.18	

Analyst-I*= Method precision;* ***Analyst-II****= Intermediate precision*

Accuracy

Accuracy (recovery) studies using a manipulated API with a known placebo dose were conducted. Three sets of triplicate samples were produced according to the recommended procedure.

Recovery Levels	Amount added (mg)	Amount recovered (mg)	% Recovered	Mean % recovery	% RSD
80% Rec-1	80.38	80.33	99.8	100	0.48
80% Rec-3	80.61	81.06	100.56		
80% Rec-3	80.94	80.66	99.65		
100% Rec-1	100.31	101.35	101.04	100.35	0.6
100% Rec-3	100.53	100.64	100.13		
100% Rec-3	101.03	100.91	99.89		
130% Rec-1	130.64	131.05	100.34	99.17	1.03
130% Rec-3	130.83	119.06	98.54		
130% Rec-3	130.67	119.03	98.63		

Range

The analytical technique has been shown to have an appropriate degree of accuracy, Accuracy, and Linearity across the range of LOQ to 130% of the analyte concentration in the sample.

Limit of Detection and Quantification

The signal-to-noise ratio was used to calculate the lower limits of detection (LOD) and quantification (LOQ). This threshold was established as the concentration below which detection signal was not discernible (3:1 signal to noise ratio) and quantification signal was not discernible (10:1 signal to noise ratio).

S. No	Drug name	LOD(µg/mL)	LOQ(µg/mL)
1	Pitavastatin	0.0033	0.0088

Stability in analytical solution

The shelf life of a sample solution was determined by a laboratory investigation. Acceptance criterion: The difference in% assay should not be higher than 3.0 from the original. since the first analysis and subsequent analyses reveal no significant differences.

S. No	Drug name	Time	% Assay	Difference
1	Pitavastatin	Initial	99.3	1.7
		About 36 h	97.5	

Robustness

Instrumental conditions such as flow rate (10%), column oven temperature (5%), detection wavelength (5nm), organic content in mobile phase (3%), and pH of buffer in mobile phase (0.3 units) were studied to determine the method's robustness. Under each condition, a duplicate sample solution and a single injection of the reference solution were injected, and the assay was computed. Table provide the mean, standard deviation, and % RSD for each set and system appropriateness values, respectively. The method's robustness is measured by the overall values of the percent RSD between set I data and data at each variable condition (Acceptance criterion: Overall RSD should not be greater than 3 percent).

Set I	Control (Proposed method)
Set II	Variation in flow rate (-10%)
Set III	Variation in flow rate (+10%)
Set IV	Variation in wavelength (l -5 nm)
Set V	Variation in wavelength (l+5 nm)
Set VI	Variation in organic content in mobile phase (-3%)
Set VII	Variation in organic content in mobile phase (+3%)
Set VIII	Column oven temperature (-5°C)
Set IX	Column oven temperature (+5°C)
Set X	Variation in pH of buffer in mobile phase (-0.3 units)
Set XI	Variation in pH of buffer in mobile phase (+0.3 units)

S. NO	Set-I	Set-II	Set-III	Set-IV	Set-V	Set-VI	Set-II	Set-VIII	Set-IX	Set-X	Set-XI
1	3.99	3.89	4.03	3.94	3.89	4.07	3.99	4.01	4.06	3.96	4.09
3	4.08	3.99	3.95	4.03	4.04	3.99	3.95	3.96	3.98	4.04	3.99
3	3.96	3.99	3.89	3.99	4.09	3.93	4.06	3.99	3.94	4.06	4.06
4	4.03	-	-	-	-	-	-	-	-	-	-
5	3.89	-	-	-	-	-	-	-	-	-	-
6	3.96	-	-	-	-	-	-	-	-	-	-
Mean	3.98	3.96	3.95	3.98	4.01	3.99	4	3.99	3.99	4.03	4.05
SD	0.06	0.06	0.07	0.04	0.1	0.08	0.06	0.03	0.06	0.05	0.05
RSD (%)	1.61	1.44	1.65	1.01	3.6	1.88	1.39	0.63	1.53	1.33	1.37
Overall mean		3.97	3.97	3.98	3.99	3.99	3.99	3.98	3.99	4	4
Overall SD		0.06	0.06	0.05	0.07	0.06	0.06	0.05	0.06	0.06	0.07
Overall RSD (%)		1.51	1.51	1.36	1.75	1.51	1.5	1.35	1.51	1.5	1.75

System suitability values for all Robustness conditions

S. No	Drug	Retention Time(min)	Column efficiency (Number of theoretical plates)	USP Tailing	%RSD for replicate injections
1	Set-I	3	3533	1.6	0.9
3	Set-II	3.3	3961	1.4	0.7
3	Set-III	3.8	3386	1.5	0.8
4	Set-IV	3.1	3649	1.3	0.4
5	Set-V	3.1	3915	1.3	0.6
6	Set-VI	3.1	3794	1.7	1.4
7	Set-VII	3.8	3518	1.3	0.8
8	Set-VIII	3.3	3996	1	1.1
9	Set-IX	3.8	3641	1.1	1.3
10	Set-X	3.9	3015	1.3	1.1
11	Set-XI	3.1	3983	1	1.1

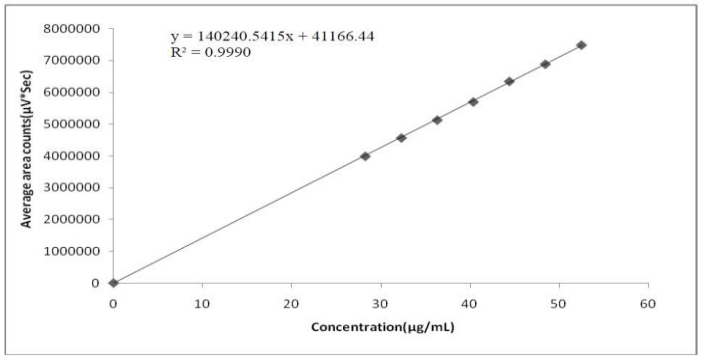
System suitability:

During the procedure's validation experiments, a standard solution was injected. The USP Tailing, column efficiency, and %RSD for duplicate injections were determined using the system appropriateness software.

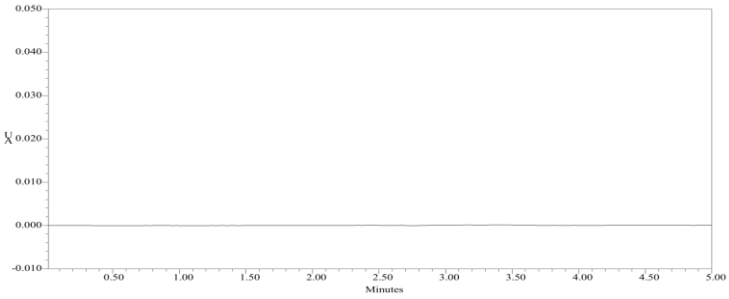
Results of system suitability

S. No	Drug	Retention Time(min)	Column efficiency(Number of theoretical plates)	USP Tailing	%RSD for replicate injections
1	Pitavastatin	3.9-3.3	3333-3156	0.9-1.4	0.3-1.4

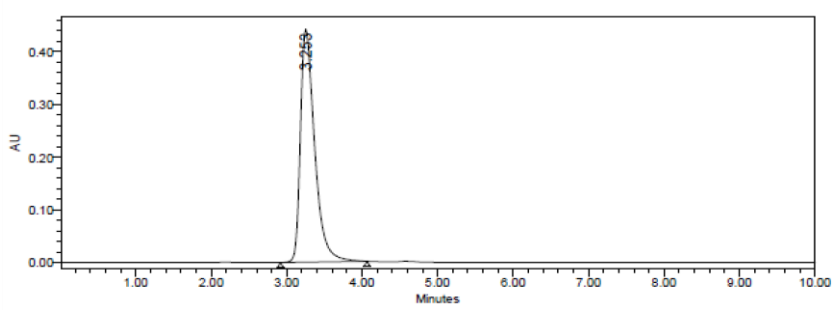
Linearity of Detector Response Graph



Blank chromatogram for the proposed method



Typical chromatogram for the proposed method



RESULTS AND DISCUSSION

PVS has been successfully separated, as seen by the chromatogram. At the analyte peak retention time, there is no interference from excipients or degrading contaminants. If there are no additional peaks that co-elute with the primary peaks, it is clear that the approach is both specific and stable, as shown by the peak purity findings obtained from the forcibly degraded samples using the given procedure. This work examined Pitavastatin chromatography on a Waters Symmetry column. Various mobile phase compositions and ratios were used to produce a robust and acceptable HPLC technique for the quantitative measurement of Pitavastatin. To achieve desirable peak shape and resolution, we conducted exploratory experiments with a variety of mobile phase compositions consisting of buffer and acetonitrile in various ratios. Research indicates that acetonitrile (35:65) (v/v) is the optimal mobile phase buffer, and 0.7 mL/min is the optimal flow rate for HPLC analysis. In order to produce a symmetric peak and avoid shifting of peak retention time, the column oven temperature was tuned at 30 °C, which results in a shorter run time but more than the limit of tailing. Using these parameters, we were able to get a single, well-defined peak for pitavastatin that was both sharp and symmetrical.

Mobile phase conditions of 80:19.8:0.3 (v/v) acetonitrile:water:triethylamine, flow rate of 1.5 ml/min, and run duration of 15 minutes were reported by H. J. PANCHAL9 et al. The suggested approach improves upon the established one by virtue of its simpler mobile phase component selection, appropriate flow rate, and shorter run duration of 6 minutes.

The lack of stability is reflected in the low detection and quantification limits reported by K. Tirumala16 et al. The suggested technique is superior to the established method in that it has benefits such as a lower detection limit (0.0088 g/mL) and a higher stability indicator (0.0033 g/mL).
Comparison of the performance characteristics of the present method with some of the reported methods

Sr. No.	Mobile phase /Column	Flow Rate (mL/minute)	Wave length(nm)	R.T / Run Time (Min.)	Linearity (µg/mL)	Remarks
1	Acetonitrile–water–triethylamine 80:19.8:0.3 (v/v)/ (C18 (Xttera); 350 x 4.6 mm;	1.5	338	5.7/15.0	0.1 - 3.5	Very narrow linearity range/complex mobile phase
2	Buffer: acetonitrile in the ratio of 30:80 v/v / (C18 (Xttera); 150 x 4.6 mm; 5µm)	1.3	348	4.1/10.0	35-150	Lacking stability indicating nature
3	Buffer pH3.0: acetonitrile 35:65 (Symmetry C18 (Xttera); 150 x 4.6 mm; 5µm)	0.7	345	3.3/10.0	0.01 - 53	Stability indicating method Wide linearity range (PDA detector)

System performance:
Several statistical variables, including relative retention, theoretical plates, and peak asymmetry, have been computed using the observed readings and documented in table 3.13 to determine the system appropriateness for each suggested approach.

Method validation:
All other parameters of this approach were also computed and described, and the methods were confirmed using linear fit curves since the UV absorption spectrum was used for final detection.

Parameters fixation:
When crafting a procedure, we studied the impact of different factors systematically by changing only one at a time while keeping the others constant. The following research was done to help with this.

Mobile phase characteristics:
The author has performed numerous experiments, changing only one variable at a time, such as the percentage of organic phase in the mobile phase, the pH of the aqueous phase, the total pH of the selected mobile phase, and the flow rate, to achieve the desired sharp peaks and baseline separation of the components. The suggested methods include the optimal circumstances that were attained.

Detection characteristics:
Standard solution was added independently to each solution, and all solutions were evaluated according to the methods to determine whether the PVS medication had been linearly eluted from the column. Graph 3.1 shows the results of a calculation done on the peak average area counts of each component. Graphics were used to show the Linear fit of the system. The slope, intercept, and correlation were calculated using least squares regression. The data is shown in Table 3.4.

Precision:
Six duplicates of a standard dose of medication were really determined, and the peak area was determined independently to establish the method's accuracy. Table 3.6 displays the results of a calculation of the percent relative standard deviation for PVS. Table 3.7 displays the results of an analysis performed to establish the assay's accuracy in terms of intra- and inter-day variation in the peak regions of the drug solutions. The findings were expressed as an overall relative standard deviation (%RS).

Stability analysis of formulations:
Tablets containing PVS were examined both immediately and after a few hours to see whether the suggested approach was suitable for the analysis of pharmaceutical formulations. Table 3.10 displays the outcomes.

Recovery studies:
Using the suggested approach, samples of the active pharmaceutical component were spiked at 80%, 100%, and 130% of the intended concentration, and then recovery trials were performed on the known quantity of placebo

Conclusion:

Analytical technique development and validation are essential steps in the pharmaceutical development process. Several critical elements contribute to success in these areas, which in turn aids in regulatory compliance. One of these criteria is the amount of expertise present in the development and validation team, both in terms of its individual scientists and as a whole. In this talk, we'll go over the steps used to create and verify an analytical technique (High performance liquid chromatography, or HPLC) for use with multi-ingredient pharmaceuticals. Quality control labs employ the approved test techniques developed via these procedures to verify the identification, purity, potency, and efficacy of pharmaceuticals.

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