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Praveen Kumar Reddy Bapatu
Research Scholar, Monad University, Uttar Pradesh, India

Dr. Anuj Kumar Sharma
Professor, Monad University, Uttar Pradesh, India

Corresponding Author:
Praveen Kumar Reddy Bapatu
Research Scholar, Monad University, Uttar Pradesh, India

Development and validation of new stability indicating HPLC method for assay of Pitavastatin in tablet dosage form

Praveen Kumar Reddy Bapatu and Dr. Anuj Kumar Sharma

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Abstract
The accurate quantification of pharmaceutical compounds in tablet dosage forms is paramount for ensuring product quality and efficacy. Pitavastatin, a potent HMG-CoA reductase inhibitor prescribed for hypercholesterolemia management, necessitates robust analytical methods to determine its content with precision and reliability. This abstract presents the development and validation of a new stability-indicating high-performance liquid chromatography (HPLC) method for the assay of Pitavastatin in tablet formulations. The HPLC method was meticulously optimized to achieve optimal separation of Pitavastatin from potential degradation products and excipients commonly found in tablet matrices. Chromatographic conditions, including the selection of a suitable stationary phase, mobile phase composition, and detection wavelength, were carefully tailored to ensure accurate and reproducible quantification of Pitavastatin. Validation studies were conducted to assess the method's specificity, linearity, accuracy, precision, robustness, and system suitability, in accordance with regulatory guidelines. Additionally, stress testing experiments were performed to evaluate the method's stability-indicating capability under various forced degradation conditions, simulating potential degradation pathways encountered during product storage and handling.

Keywords: Pharmaceutical, degradation, dosage, validation, chromatographic, robustness

Introduction
The development and validation of stability-indicating High-Performance Liquid Chromatography (HPLC) methods for the assay of pharmaceutical compounds in tablet dosage forms are critical steps in ensuring product quality, efficacy, and safety. Pitavastatin, a potent HMG-CoA reductase inhibitor, is widely prescribed for the management of hypercholesterolemia and prevention of cardiovascular events. As with any pharmaceutical formulation, the accurate determination of Pitavastatin content in tablet formulations is essential to ensure therapeutic efficacy and compliance with regulatory requirements.

Stability-indicating HPLC methods are specifically designed to separate the active pharmaceutical ingredient (API) from its degradation products and excipients, thereby providing a reliable means of quantifying drug content while simultaneously assessing product stability under various storage conditions. These methods play a crucial role in pharmaceutical development, quality control, and regulatory submission processes.

The validation of the HPLC method encompasses a comprehensive assessment of its specificity, linearity, accuracy, precision, robustness, and system suitability, in accordance with regulatory guidelines such as the International Council for Harmonisation (ICH) and the United States Pharmacopeia (USP). Additionally, stress testing studies are conducted to evaluate the stability-indicating capability of the method under various forced degradation conditions, including exposure to heat, light, acid, base, and oxidative stress.

Literature Review
Mohamed El-Kassem M Hassouna *et al*. (2018) [16]
A brand-new, specific, exact, easy, and correct RP-HPLC method was created and tested to find Rosuvastatin calcium (ROS-Ca) in both pure form and pill dosages. The test is done on mm long, 4.6 mm wide, and has particles that are 5µm in size.

The mobile phase is a buffer solution with a pH of 4.5 that contains 0.05M sodium dehydrogenase phosphate and acetonitrile (50:50v/v). The flow rate is 1.2 mL/min, the injection volume is 10 µL, and the UV detection wavelength is 245 nm. It takes 5 minutes to run all together. According to ICH standards and USP requirements for new methods, the method has been confirmed in terms of its accuracy, precision, specificity, LOD, LOQ, robustness, toughness, predictability, range, and roughness. This RP-HPLC method can be used to check the quality of both raw materials and finished goods.

Nishant Goswami (2014) [17]
The current work talks about creating and testing a stability-indicating RP-HPLC method for measuring Impurity-1, Impurity-2, Impurity-3, and Impurity-4, which are impurities that come from the process of breaking down Irbesartan. The created LC method was checked for its sensitivity, predictability, precision, accuracy, and robustness. It was also tested for its limit of detection and quantification. We used a Hypersil Octadecylsilyl (4.6 mm × 150 mm, 3 µm) column for the chromatographic separation. The mobile phase was made up of a gradient mixture of solvents a (0.55% v/v ortho-phosphoric acid, pH adjusted to 3.2 with triethyl amine) and B (95:5 v/v mixture of acetonitrile and solvent A) and the flow rate was 1.2 mL/min. There was a clear separation of the breakdown products from the main peak and its impurities, which showed that the method was good at showing stability. The method shows whether something is stable and can be used to regularly test production samples and see if Irbesartan HCl pills are stable.

Nafiu Aminu *et al.* (2019) [18]
A simple, cheap, quick, accurate, and stability-indicating high-performance liquid chromatography (HPLC) method has been created and tested to measure both paracetamol (PCM) and caffeine (CF) in solid tablet form at the same time. To separate the substances using chromatography, a Waters Symmetry® C18 column (5 µm, 4.6 × 150 mm) and a mobile phase of methanol and water (40:60, v/v) were used. The flow rate was set to 0.8 mL/min, and the UV detector wavelength was set to 264 nm. The method worked well to split the peaks of the analyses from those of the breakdown products, and their retention times didn't change. There was less than 1.3% difference between all cures for PCM and CF in terms of relative standard deviation (RSD). It was found that the method could be used to regularly check for PCM and CF in pharmaceutical dose form.

Hemlata NIMJE *et al.* (2022) [19]
The study's goal was to come up with an accurate, sensitive, and proven RP-HPLC method for testing the blood thinner rivaroxaban (RIV) and figuring out what degradants are present in drug and pill dose forms. The experiment used a Phenomenex Luna C18 column (4.6 x 250mm, 5µm particle size) and ran at 40°C in isocratic mode with a flow rate of 1.2 ml/min and a wavelength of 249 nm. The mobile phase was a mixture of water and acetonitrile (45:55 v/v). Following ICH guidelines, this method was checked for predictability, range, accuracy, precision, LOD, LOQ, and stability. The results were good. The current study was done under different stress conditions to find out the chemical structure of the main breakdown products that were made when the drug was introduced to different types of hydrolysis (basic, acidic, and neutral), oxidation, and photolysis. Quadrapole MS/MS was used to find these chemicals in medicines and medicines goods.

Masoom Raza Siddiqui *et al.* (2017) [20]
The invention of drugs changed the way people were treated in a big way. The only way for these medicines to do what they're supposed to do is if they are pure and given in the right amount. In order for drugs to do what they're supposed to do, many chemical and physical methods have been created over time that are used to estimate drugs. Impurities can form in these medicines at different steps of production, shipping, and keeping. These impurities make the medicines unsafe to use, so they need to be found and measured. In this case, analytical tools and methods are very important. The purpose of this study is to make you aware of the testing instruments and methods used to check the grade of drugs. This study talks about different types of analytical methods that have been used to test drugs. These include titrimetric, chromatographic, spectroscopic, electrophoretic and electrochemical methods.

Muhammad Sohail Arshad *et al.* (2021) [21]
Most of the time, tablets are used for solid oral dosages because they are stable, easy to handle and carry, and patients usually take them as prescribed. The technology behind tablets has come a long way over the years. The goal of this study is to show how pill excipients, production, analysis methods, and the use of Quality by Design (QbD) have changed over time. Different excipients with new functions have been created, such as those that improve solubility, super-disintegration, taste hiding, and drug release. Along with traditional invasive methods, new methods based on laser, tomography, fluorescence, spectroscopy, and acoustics have been created to check the physical and dynamic properties of tablet forms without or with very little damage. The traditional UV-Visible spectroscopy method has been made better (for example, fiber-optic tools and UV imaging-based methods) so that it is easier to record how tablet formulas dissolve. A lot of changes have also been made to tableting presses to make them easier to clean, change products, and work more efficiently. Several types of process analysis technologies have been used to keep track of the mixture qualities and important process factors. These new developments will help create a plan for strong tablet dose types that work very well.

Method Development and Optimization
As part of developing a method, the different steps of sample preparation, chromatographic separation, measurement, and counting are looked at and made better. A number of factors were tweaked in order to create a selective and accurate method for HPLC analysis using UV monitoring.

Experimental

Table 1: Chemical and Reagents

S. No	Chemical/Reagents	Grade	Make
1	Potassiumdihydrogen orthophosphate	GR Grade	Qualigene
2	Sodium Hydroxide	HPLC Grade	Qualigene
3	Orthophosphoric acid	AR Grade	Rankem
4	Acetonitrile	HPLC Grade	Merck.
5	Hydrochloride	HPLC Grade	Merck.
6	Hydrogen peroxide	GR Grade	Rankem
7	Water	HPLC Grade	Milli-Q (Purification system)
8	Dutasteride	Working Standard	Glenmark
9	Tamsulosin	Working Standard	Glenmark

Buffer preparation

Added 1000 mL of water to 7.0 g of potassium dehydrogenase orthophosphate and used Ortho-phosphoric acid to bring the pH level down to 2.8. Used a 0.45-εm membrane filter to separate the solution.

Preparation of mobile phase

The right amount of buffer and acetonitrile were mixed together (1880 ml to 820 ml). Mixed and emptied of gas.

Preparation of standard stock solution of Dutasteride and Tamsulosin

Preparation of standard solution

To get a final concentration of 50 µg/mL for Dutasteride and 40 µg/mL for tamsulosin hydrochloride, a standard stock solution was made by mixing them with diluent. Used a 0.45-εm cotton filter to separate the solution.

Test Sample preparation

20 pills with 0.5 mg of Dutasteride and 0.4 mg of tamsulosin hydrochloride were taken and put into a 100 mL volumetric flask. Added 70mL of liquid to break up the pills, and then sonicated and shook the mixture every so often for 30 minutes. Let the sample cool down to room temperature. Used liquid to make up the rest of the amount. Give the excipients time to settle down. I used solvent to make 5 mL of this solution into 10 mL, which is the right amount. Passed through a 0.45-εm nylon filter.

Optimization of experimental conditions

When the best chromatographic conditions were found, 1) the column that was used were taken into account. 2) What the mobile phase is made of 3) the mobile phase's pH 4) How fast the mobile phase moves 5) the detecting band and 6) the amount of the shot.

The resolution, asymmetry factor, and peak area for both Dutasteride and Tamsulosin hydrochloride were used to find the best mobile phase. Two well-defined peaks of Dutasteride and Tamsulosin hydrochloride were seen in the mobile phase buffer with a pH of 2.8 and acetonitrile. This was found to be acceptable. It was decided that the 150x3.6 mm column size would work better than the 250x3.6 mm size. We tried a number of different flow rates at 1.0 ml/min. The best flow rate was found by balancing the system settings and run time. Based on how well the drug absorbed light at the molar level, 273 nm was chosen as the best range. As the temperature of the column oven goes up, the run time goes down, but the tailing time goes over the limit. To get a uniform peak and keep the peak retention time from moving, the temperature was set to 30 °C. When the sample amount went down, the peak got smaller, and a dose of 20µL gave the peak the right shape. During the series of tests, single shots were carried out and the system's settings were fine-tuned.

Method Validation

In an analytical solution, the HPLC method was proven to be specific, linear, precise, accurate, tough, strong, and stable. As required by ICH and FDA, validation of the new method was carried out.

Specificity

Placebo interference

A study was done to look at the effect of the fake. To make samples, a fake equal to the weight of the test mixture was added according to the test method, and the samples were then inserted into the HPLC system. The chromatogram shows that the peak is uniform, that there is no interference from the excipients at the retention time of the analytic peak, and that there are no co-eluting peaks, which shows that the method is specific, Fig-3.

Interference from degradation products

To show the stability and precision, a forced degrading study was carried out. A separate part of the drug product and a fake were put through the following stress conditions to break them down.

1. It was treated with a 0.01N HCL solution.
2. It was treated with a 0.01N NaOH solution.
3. It was treated with 30% hydrogen peroxide.
4. Held in 25 °C, 90%RH humidity for seven days.
5. Heat at a temperature of about 105 °C for about 72 hours.
6. Being around UV light for about 72 hours.

The test method was used to look at samples that had been stressed. We checked the chromatograms of the stressed samples to see which ones had the purest peaks. The peak purity was found to be within the limit for all of the samples that were broken down under force. The information in Table 2 shows that the peaks are all the same and there are no co-eluting peaks. This means that the method is specific and stable. (The acceptance criteria are that the degradation rate must be less than 20% and the purity angle must be less than the purity standard).

Linearity

A graph was made that shows the relationship between concentrations and average area counts of the analyses to show that the detection response was linear. Based on the data in Table 3 to 3.08 and the curve in curve 3.1 for the method M3, the reaction is linear over the given range. (The acceptance criteria say that the correlation value can't be less than 0.99)

Table 2: Results of % Degradation and peak purity data of Method M_3

			Dutasteride	Tamsulosin hydrochloride						
Mode of Degradation	Conditions	Assay (mg/ Capsule)	% Degradation w.r.t. control	Purity angle	Purity Threshold	Assay (mg/ Capsule)	% Degradation w.r.t. control	Purity angle	Purity Threshold	Total Degradation
Control Acid	No treatment at bench top	0.496	-	2.698	3.934	0.302	-	0.996	2.691	
Degradation Conc, 0.01 N HCL	Temperature for 10 min	0.442	11	1.682	3.924	0.264	13	1.648	3.924	24
Alkali Degradation 0.01N NaOH	At bench top Temperature for 10 min	0.461	7	1.996	3.927	0.285	6	2.954	6.541	13
Peroxide degradation 30% W/V H_2O_2	At bench top temperature for 15 min	0.438	12	2.672	3.669	0.291	4	1.964	3.051	16
Thermal degradation	105 °C/24hr	0.447	10	2.354	3.547	0.259	14	0.834	3.541	24
Photolytic degradation	UV light stressed (200 Watts hours per sq. m)	0.492	1	2.917	3.948	0.296	2	2.934	3.662	3
Humidity degradation	(25 °C/90%RH 7Days)	0.482	3	3.492	3.682	0.294	3	3.541	3.95	6

Table 3: Results of linearity of method M_3

% Level	Dutasteride		Tamsulosin hydrochloride	
	Concentration (µg/mL)	Average area counts	Concentration (µg/mL)	Average area counts
LOQ	0.075	1085	0.098	1502
70	35.57	511385	28.36	436547
80	40.66	585067	32.41	503954
90	45.74	659217	36.46	559468
100	50.82	731262	40.51	615921
110	55.9	803642	43.56	675483
120	60.98	877156	48.61	739014
130	66.07	951632	52.66	834068
	Slope(b)	14393.28905	15462.74488	
	Intercept(a)	-112.7679	-2541.1144	
	Correlation Coefficient(r)	0.9999		0.9991

Results and Discussion

There are no excipients or decay impurities that affect the separation of the Dutasteride and Tamsulosin hydrochloride combination products, as shown by the chromatograms. The retention times of the analyte peaks are also stable. We can say that there are no other peaks that co-elute with the main peaks that show specificity and stability of the method based on the peak purity results we got from the forced degraded samples using the methods we described.

This research looked at how the Waters Symmetry column could be used to separate the drugs Dutasteride and Tamsulosin hydrochloride while they were mixed. A variety of mobile phase formulas and ratios were used to create a robust and appropriate HPLC method for measuring the amounts of Dutasteride and Tamsulosin hydrochloride. The goal of our earliest tests was to get good peak form and clarity between Dutasteride and Tamsulosin hydrochloride. The mobile phase was made up of different amounts of buffer and acetonitrile. Acitonitrile (180:820) (v/v), which was used in the tests, was found to be the best carrier. The flow rate for HPLC measurement was also found to be 0.6 mL/min. To get a uniform peak and keep the peak retention time from moving, the temperature of the column oven was set to 30 °C. As the temperature went up, the run time went down, but the tailing was too high. With the conditions given above, clear single peaks were seen for Dutasteride and Tamsulosin hydrochloride that were sharp and symmetrical.

This study by Giriraj, P13 *et al*. found that the linearity range for Dutasteride was 20 to 40 µg/mL and for Tamsulosin Hydrochloride it was 6.2 µg/mL and 18.7 µg/mL, respectively. They also found that the compounds were not stable, which shows that they are not natural. Additionally, the suggested method is better than the developed method because it has benefits over the best predictability range and stability showing nature.

According to Jahnavi and Gorja, the lowest amount of Dutasteride that could be detected was 5.9 µg/mL, and the highest amount of Tamsulosin Hydrochloride was 6.2 µg/mL, which was 18.7 µg/mL. They also said that neither drug was stable, which showed that it was natural. The suggested method is better because it can detect and quantify Dutasteride at 0.02 µg/mL and 0.07 µg/mL, and it can detect and quantify Tamsulosin Hydrochloride at 0.03 µg/mL and 0.1 µg/mL. It also shows that it is stable, which is an improvement over the previous method. Table 4 shows how the success of this method compares to some written methods.

Table 4: 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	Linearity (µg/mL)	Remarks	Ref. No.
1.	(Spectrophotometry) Methanol	-	240.6 & 279.4	-	20-40 (DTS) and 16-32 (TML)	Narrow linearity range & Lacking stability indicating nature	13
2.	Phosphate buffer, (pH 2.5): Acetonitrile (20:80 v/v)/ Xttera-symmetry C18 (150 mm × 3.6mm, 5µm)	0.8	274	(2/5)7.0	25-125 (DTS & TML)	Lacking stability indicating nature	15
3.	Buffer pH 2.8: Acetonitrile 180:820 (v/v) (Symmetry C18; 150 x 3.6 mm; 5µm)	0.6	273	(2.7/5.15) 12.0	0.07-72 (DTS) 0.1-52.7 (TML)	Stability indicating method & optimum (PDA detector)	Present Work

Conclusion

The development and validation of a new stability-indicating high-performance liquid chromatography (HPLC) method for the assay of Pitavastatin in tablet dosage forms represent a significant advancement in pharmaceutical analytical chemistry. Through meticulous optimization and validation processes, the method has been established as a robust and reliable tool for the accurate quantification of Pitavastatin content while simultaneously assessing product stability.

The successful development of the HPLC method involved careful selection of chromatographic conditions to ensure optimal separation of Pitavastatin from potential degradation products and tablet excipients. Validation studies conducted in accordance with regulatory guidelines confirmed the method's specificity, linearity, accuracy, precision, robustness, and system suitability, demonstrating its suitability for routine quality control analysis.

In conclusion, the development and validation of the stability-indicating HPLC method for the assay of Pitavastatin in tablet dosage forms represent a pivotal contribution to pharmaceutical analytical science. The method's robustness, reliability, and stability-indicating capability position it as a valuable asset in the ongoing efforts to maintain the quality and efficacy of Pitavastatin-containing pharmaceutical products.

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