NEW METHOD FOR THE SIMULTANEOUS ESTIMATION OF FENOFIBRATE AND PITAVASTATIN BY USING RP-HPLC IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract

The aim of this study is to create and validate a fast, easy-to-use, affordable, sensitive, and accurate method for measuring Fenofibrate and Pitavastatin in bulk and pharmaceutical products using Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC). A Luna C18 column was used with a running phase composed of 0.1% trifluoroacetic acid: acetonitrile (30:70 v/v) at a flow rate of 1.0 ml/min. UV detection was used at a wavelength of 240 nm. Pitavastatin and Fenofibrate correlation coefficients were found to be 0.999 over a concentration range of 5-30 µg/ml and 168.75-1012.5 µg/ml, respectively. Pitavastatin and Fenofibrate have respective retention times of 3.310 and 2.126 minutes. The run time for separating Pitavastatin and Fenofibrate peaks was 5 minutes. This method, proposed as a regular analysis and quality control tool for medications that contain these active drugs either individually or in combinatio, was evident to be a suitable one.

Keywords: *HPLC, Pitavastatin, Fenofibrate, Development, Validation.*

1. Introduction

The fibrate[1] class used to treat abnormal blood lipid levels. It is less commonly used compared than statins[2-3] because it treats a different type of cholesterol abnormality to statins. While statins have strong evidence for reducing heart disease and death, there is evidence to suggest that fenofibrate also reduces to the risk of heart disease and death. However, this seems only to apply to specific populations of people with elevated triglyceride[4] levels and reduced high-density lipoprotein (HDL) cholesterol[5]. Its use is recommended together with dietary changes. Common side effects include liver problems, breathing problems, abdominal pain, muscle problems, and nausea[6]. Serious side effects may include toxic epidermal necrolysis[7], rhabdomyolysis[8], gallstones[9], and pancreatitis[10]. Use during pregnancy and breastfeeding is not recommended. It works by multiple mechanisms.

Pitavastatin (usually as a calcium salt) is a member of the blood cholesterol lowering medication class of statins. Like other statins, it is an inhibitor of HMG-CoA reductase[11], the enzyme that catalyses the first step of cholesterol synthesis. Common statin-related side effects (headaches, stomach upset, abnormal liver function tests and muscle cramps) were similar to other statins. However, pitavastatin seems to lead to fewer muscle side effects than certain statins that are lipid-soluble, as a result of the fact

that pitavastatin is water-soluble (as is pravastatin, for example). One study found that coenzyme Q_{10}[12] was not reduced as much as with certain other statins (though this is unlikely given the inherent chemistry of the HMG-CoA reductase pathway that all statin drugs inhibit). As opposed to other statins, there is evidence that pitavastatin improves insulin resistance in humans, with insulin resistance assessed by the homeostatic model assessment (HOMA-IR) method. Hyperuricemia or increased levels of serum uric acid have been reported with pitavastatin. Simultaneous determination of Fenofibrate and Pitavastatin using HPLC was found in the current study.

Till now, there is no HPLC reports available for the estimation of Pitavastatin and Fenofibrate.

2. Experimental Study

Solutions and Reagents

The pure Pitavastatin and Fenofibrate used in this study was provided by Glenmark Pharmaceutical Private Ltd., located in Andheri (E), Mumbai, India (99.7-99.9 percent purity). Other reagents, including acetonitrile, trifluoroacetic acid, and water, were obtained from Merck (India) Ltd. in Worli, Mumbai, India, and were of HPLC grade.

Collection of instruments

Waters alliance liquid chromatography (model 2695) monitored with empower 2.0 data handling system and a detector of photo diode array (model 2998) was used for this study.

Buffers are chosen

An easy, inexpensive, and appropriate acidic buffer was chosen, such as 0.1 percent trifluoroacetic acid (Observed pH-2.3).

Step of mobility

For Standard review, the mobile step was 0.1% TFA buffer in a 30:70 (v/v) acetonitrile mixture and was degassed beforehand. A mobile phase chosen to produce well-defined peaks with a low tailing factor (2.0) and a plate count of over 2000 was selected.

Prepare the diluent

The diluent's mobile process was set up.

Conditions of Chromatography

For the HPLC experiments, a Luna C18 (150 x 4.6 mm, 3.5 µm) was used. The elution was conducted with isocratic conditions using acetonitrile: TFA (0.1% volume) (70:30 by volume) at a flow rate of 1.0 ml/min. The injection volume was 10 µl, and the run time was 5 minutes, with the column temperature set to room temperature and the absorbance measured at 240 nm (Because maximum absorbance was observed at this wavelength. So, this was selected as wavelength).

Standard Solution Preparation

To dilute 20 mg of Pitavastatin and 675 mg of Fenofibrate, measure out the drug and transfer it to a 100 mL volumetric flask, then add approximately 70 mL of a diluent and sonicate for 30 minutes to dissolve it, after that add more diluent to the total amount. Further dilute 5 mL to 50 mL with diluents.

Sample Solution Preparation

Measure out 254 mg of sample and transfer it to a 100 mL volumetric flask, then add approximately 70 mL of a diluent and sonicate for 30 minutes to dissolve it, after that add more diluent to the total amount.

Validation Process[13-21]

System Precision

The system's performance has been validated through assessment of device suitability parameters. Limits were found to be met for a variety of parameters, including plate count, tailing, and RSD percentage.

Specificity

Being able to identify and test a given analyte in the presence of other elements required to be combined in the Standard and the standard solution is known as specificity. Blank Standards and those with Pitavastatin and Fenofibrate will be tested using chromatograms.

Accuracy

Being close to the real meaning of the technique is what defines accuracy. Three concentrations will be used to test the recovery trials. The drug's quantity, percentage of recovery, and standard deviations were calculated after every injection at each level.

Precision

It is the level of agreement between the various test results that determines the precision of the analytical methodology. Researchers examined the effects of sampling a homogeneous population more than once. The current process was evaluated in terms of its ability to provide repeatable, intraday, and inter-day results. It was examined by sampling the materials on the same day and over the course of different days.

Linearity

Linearity is the feature of analytical process which allows for a direct proportion of analytical results in response to a certain concentration of the analyte in the Standard. A total of seven series of standard solutions were selected for the assessment of the linearity spectrum. The calibration curve was drawn by comparing regular solution concentration with peak area. Using the least square method, the slope, intercept, and coefficient of correlation were calculated.

Forced Degradation

The peaks in the chromatogram should agree. ICH guidance Q1 (A) R2 was performed in conjunction with stress degradation experiments. The peaks of degradation should be well distanced and at least 1.0 resolution between peaks. For the largest peaks to go over, a separation must occur. A degradation of around 20 percent has been attained via several various stress conditions like acid, alkali, peroxide, reduction, thermal and photo in what is known as a forced degradation experiment.

Robustness

Robustness refers to a procedure's resistance to small process parameter changes, as well as its reliability in normal operation. An organic solution was introduced into the HPLC system for a robustness analysis, and the chromatographic settings (such as flow rate and mobile-phase organic content) were modified. The separation factor, retention time, and peak asymmetry were determined by evaluating the effects of altered parameters.

3. Results and Discussion

The aim of this study is to establish a single isocratic HPLC method for the simultaneous quantification of Pitavastatin and Fenofibrate in bulk and pharmaceutical dosage forms that is reliable, precise, and cost effective. According to the UV spectra of these compounds, an appropriate wavelength for simultaneous estimation of two drugs was chosen.

Optimization of the method

Using buffers (0.1% orthophosphoric acid, 0.1% formic acid, 0.1% triethylamine) and acetonitrile as mobile phase different trials were conducted in isocratic and gradient modes. Various stationary phases including phenyl, biphenyl, amino, C4, and C8, were

used to test the system. The resolution and retention times were improved by changing the mobile step composition at each trial. In the end, the separation was achieved using a Luna C18 column (150mm x 4.6mm, 3.5µm) and a mobile phase of 0.1% TFA: acetonitrile (30:70 v/v) with a flow rate of 1.0 ml/min and UV detection at a wavelength of 240 nm. The entire performance lasted five minutes. Conditions for optimized chromatography are provided in table 1.

System Suitability

To attain results, the following device suitability parameters were established after six consecutive injections of normal solution: theoretical plate number, time, peak area, tailing factor, and resolution. The chromatogram in Figure 1 was the representative of the suitability results detailed in table 2.

Table 1. Method suitability conditions

Parameter	Suitable conditions
Column	Luna C_{18} (150 x 4.6 mm, 3.5 µ)
Moving Phase	0.1% Tri fluoro acetic acid: Acetonitrile (30:70 v/v)
Volume of injection	10 µl
Stream rate	1.0 mL/min
Temperature of column	25°C
Wavelength	240 nm
Time duration	5 minutes
Retention time of Pitavastatin	3.310 min
Retention time of Fenofibrate	2.126 min

Table 2. Results of system suitability

Parameter	Pitavastatin	Fenofibrate
Number of plates	4598	91814
Tailing	1.02	0.89
Resolution	5.02	
Peak elution time	3.310	2.126
% RSD	0.72	1.43

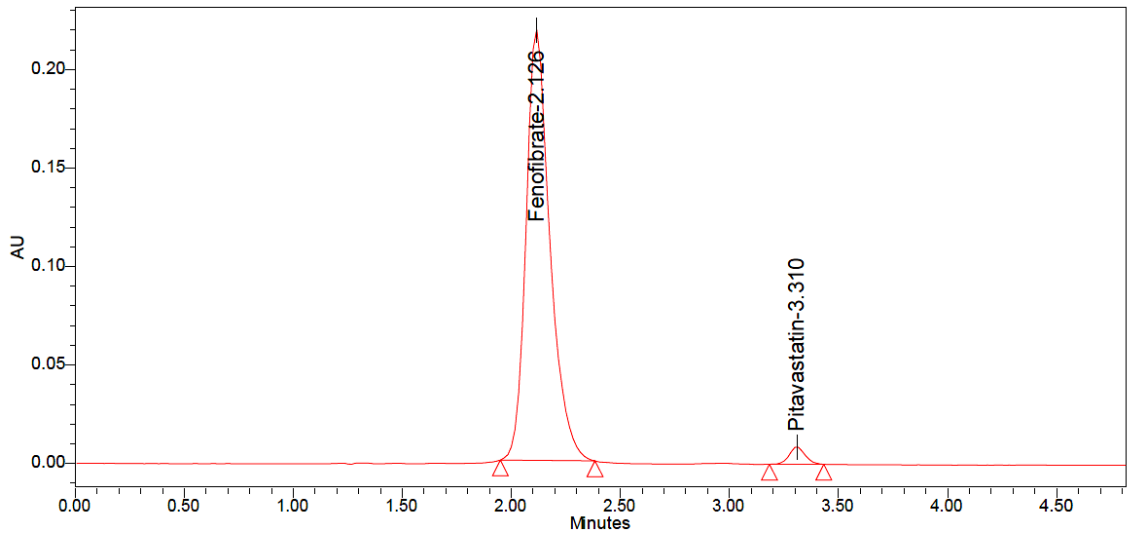

Figure 1. Chromatogram of standard

Specificity

There was no participation from Pitavastatin and Fenofibrate at the elution time. As seen in Figure 2, the blank chromatogram is present.

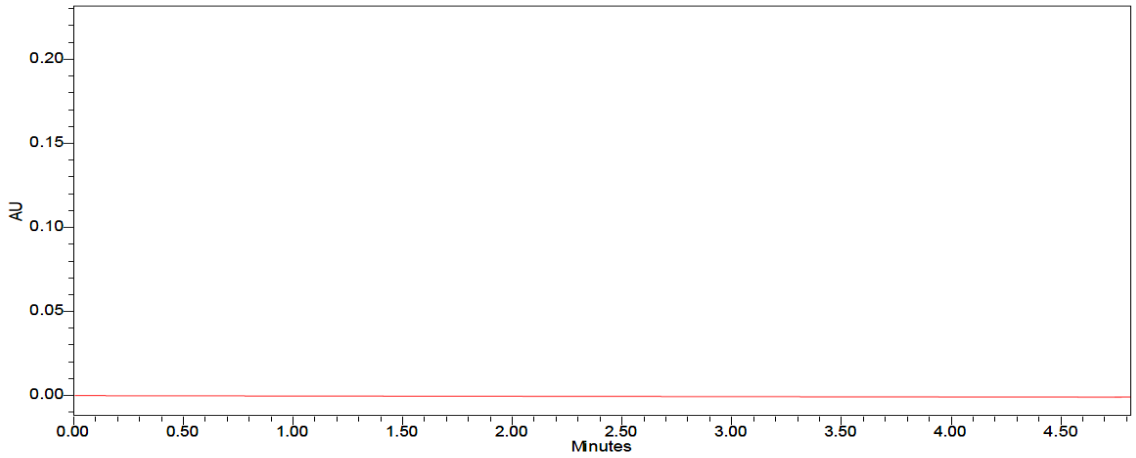

Figure 2. Chromatogram of blank

Linearity

By using a calibration curve to determine the linearity of the area of peak, its corresponding concentration was discovered. From this graph, it appears that the range of 168.75-1012.5 µg/mL of Fenofibrate and 5-30 µg/mL of Pitavastatin had a straight line. Linearity results were demonstrated in table 3.

Table 3. Results of linearity

S. No	Fenofibrate		Pitavastatin	
	Concentration (µg/mL)	Area	Concentration (µg/mL)	Area
1	168.75	628453	5.00	24128
2	337.50	1254478	10.00	45457
3	506.25	1865472	15.00	69417
4	675.00	2512635	20.00	92521
5	843.75	3114581	25.00	115589
6	1012.50	3729962	30.00	138827

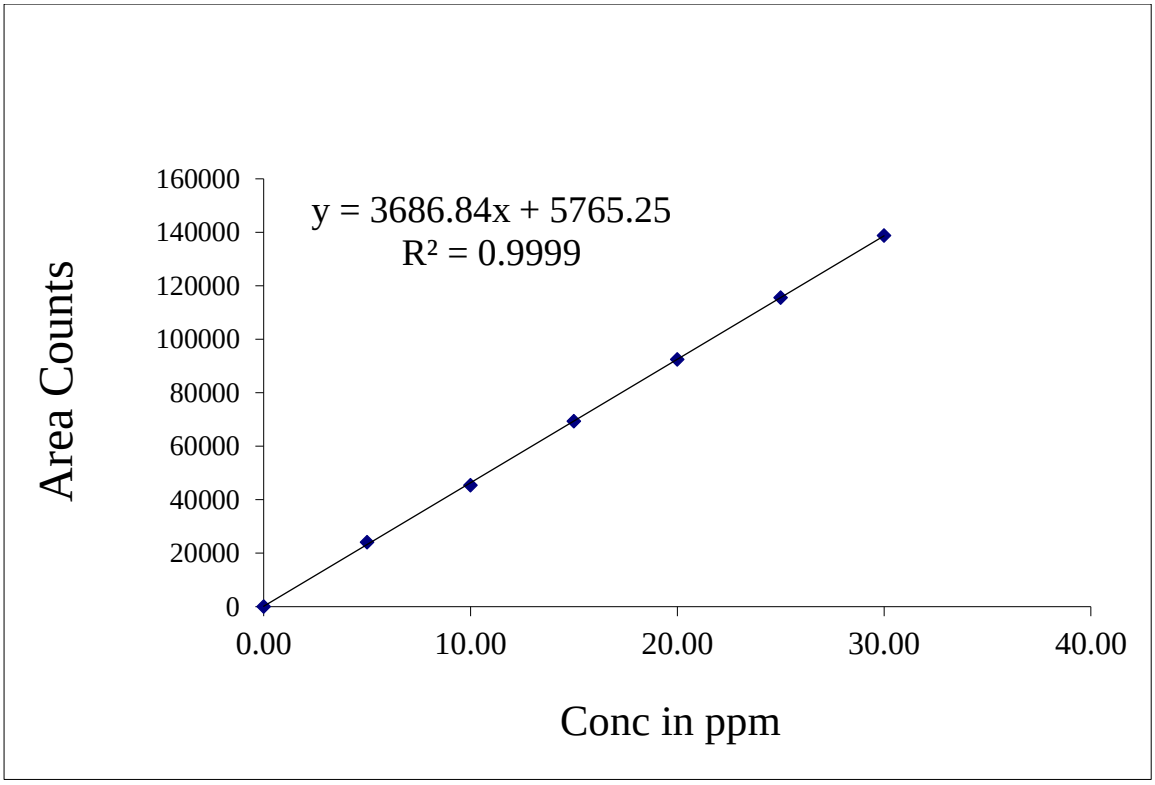

Figure 3. Calibration plot of Fenofibrate

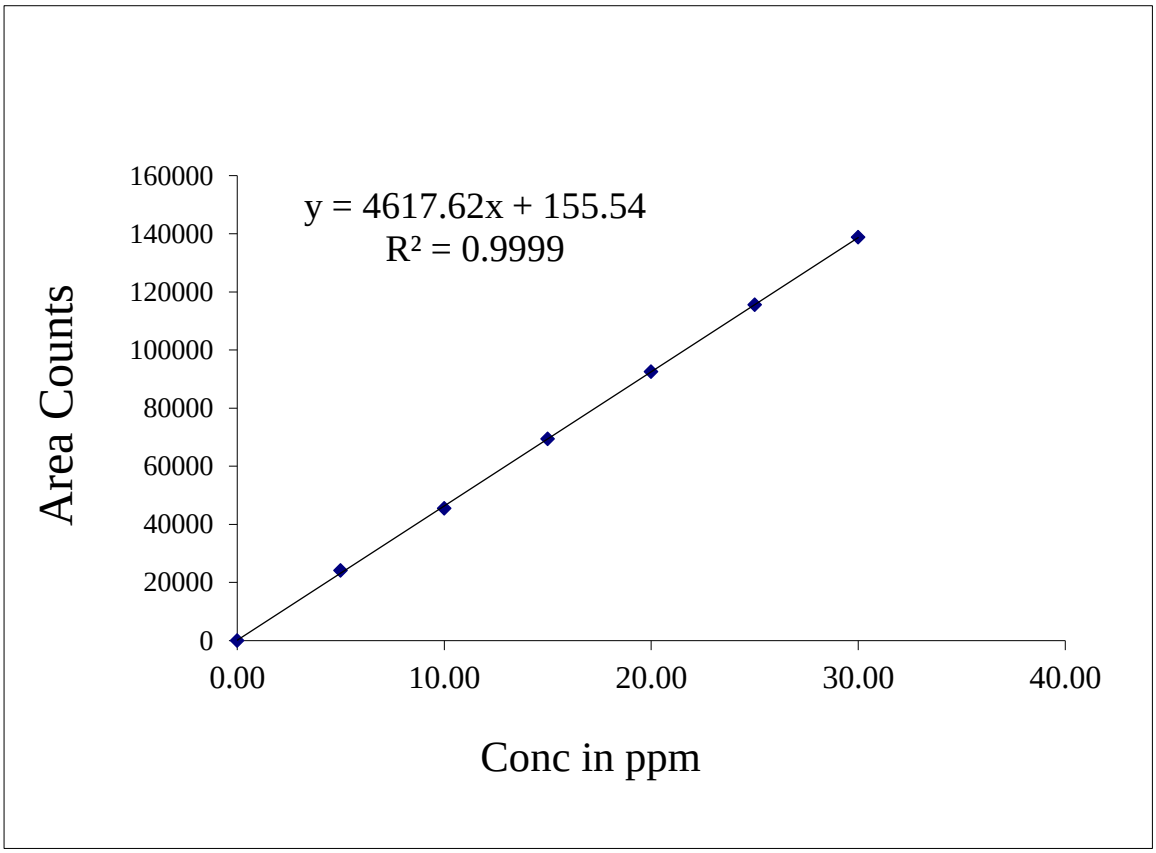

Figure 4. Calibration plot of Pitavastatin

Precision

Intraday and intermediate precision variances were assessed in relation to the procedure's accuracy. The samples were examined six times on the same day to obtain intraday results for Pitavastatin and Fenofibrate. The system's intermediate precision was explored by analyzing data in the same laboratory using a variety of examiners and tools. It is very accurate, with an RSD percentage of less than 2%. The process was precise, yielding the best drug recoveries at each additional concentration. Table 4 shows the method precision results.

Table 4. Outcomes of method precision

S. No.	Fenofibrate		Pitavastatin	
	Area	% Assay	Area	% Assay
1	2559715	99.5	92128	98.8
2	2580789	100.2	92087	98.9
3	2569223	100.4	92478	99.5
4	2545136	99.6	92687	100.6
5	2569054	100.7	92713	98.4
6	2551732	99.3	92174	98.2
Mean	2562608	99.95	92378	99.07
Std. dev	13021.409	0.561	285.133	0.876
% RSD	0.51	0.56	0.31	0.88

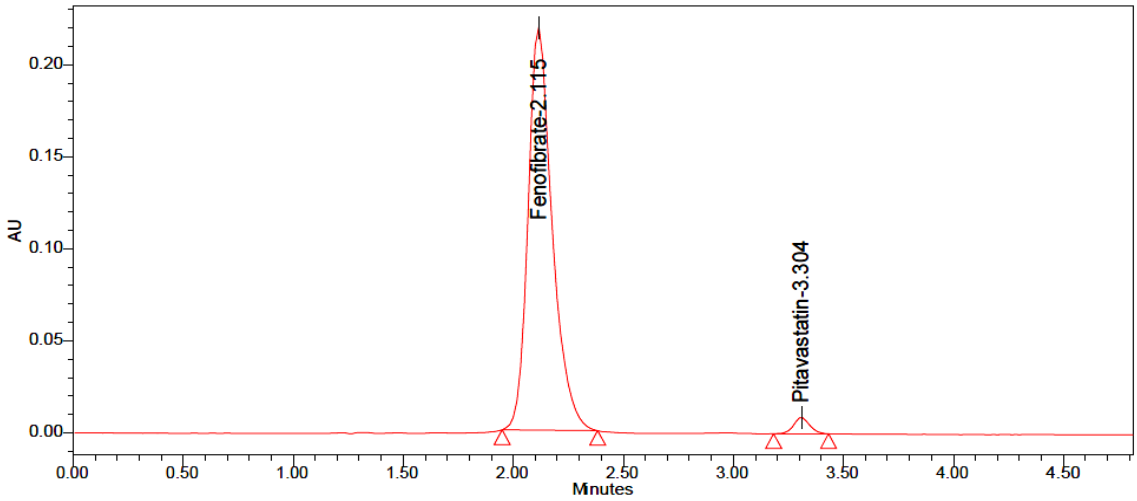

Figure 5. Chromatogram of method precision

Intermediate Precision (Ruggedness)

Intermediate precision results were shown in table 5.

Table 5. Results of intermediate precision

S.No.	Fenofibrate		Pitavastatin	
	Area	% Assay	Area	% Assay
1	2562738	99.5	92178	100.7
2	2575787	100.1	92295	99.9
3	2566247	100.4	92248	101.2
4	2549118	99.7	92418	101.1
5	2559097	99.6	92645	100.6
6	2556718	99.1	92354	98.5
Mean	2561618	99.73	92356	100.33

Std dev	9056.026	0.459	163.974	1.009
% RSD	0.35	0.46	0.18	1.01

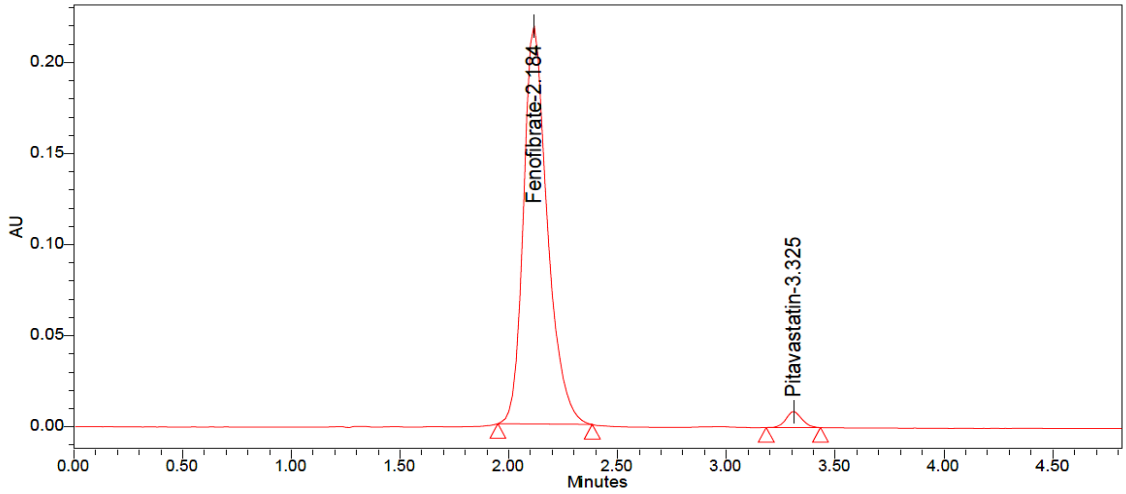

Figure 6. Chromatogram of intermediate precision

Accuracy

By measuring the recovery experiments at three stages, the method's precision was reached (50 percent, 100 percent, and 150 percent). APIs were made with concentrations of Pitavastatin of 25, 50, and 75 micrograms/mL and Fenofibrate of 2.5, 5 and 7.5 micrograms/mL. For each stage of the spike, the test solution was injected three times, and the assay was performed in accordance with the test process. In addition to being able to determine the percentage of recovered data, the mean and relative standard deviations have also been found. The strategy was effective because the recovery values fell within the target range. Table 6 presents the accuracy results.

Table 6.Results of accuracy

Accuracy	Amount of Fenofibrate	% Recovery	Amount of Pitavastatin	% Recovery
50*	33.8	99.6	1	99.1
100*	67.5	100.7	2	98.7
150*	101.3	98.6	3	98.3

* Results are mean recovery of three sample preparations

LOD and LOQ

The concentration level at which the analytes are reliably detected and quantified is the limit of detection and quantification. Pitavastatin and Fenofibrate had a LOD concentrations of 0.06 µg/ml, 2.02 µg/ml and their S/N values of 3, 3. The LOQ concentrations of Pitavastatin and Fenofibrate were 0.2 µg/ml, 6.75 µg/ml, and their S/N values were 10, 10. (S/N is the ratio of signal to noise).

Robustness

To ensure the robustness of the chromatographic technique, the researchers evaluated flow rate and the composition of the mobile phase. By changing the flow rate and mobile phase ratio, the area of drugs changes. So, the percentage of relative standard deviation changes. Here in Table 7 (robustness results) the %RSD values are in within the acceptable limit.

Table 7. Outcomes of robustness

Parameter	% RSD of Fenofibrate	% RSD of Pitavastatin
Flow (0.8 mL/min)	0.65	0.49
Flow (1.2 mL/min)	0.81	1.35

Organic phase (77:23)	1.16	0.75
Organic phase (63:37)	0.67	0.43

Forced Degradation

The proposed approach can be used for successful evaluations of release and stability tests, and it can be called a stability preferable technique. Acid, Alkali, oxidation, reduction, photo, and thermal degradation are all included in the ICH-required forced degradation analysis. The chromatograms show that the selected drugs remained stable under the stress conditions, despite the presence of degraded peaks. Results of forced degradation were given in table 8.

Acid degradation

A volume of 1 ml sample stock solution was transferred to a volumetric flask with a capacity of 10 ml, to which 1 ml of 1N HCl was added and left to stand for 15 minutes. After 15 min add 1 ml of 1N NaOH and make up to the diluent mark. Filter the solution using syringe filter and injected into HPLC system.

Alkali degradation

A volume of 1 ml sample stock solution was transferred to a volumetric flask with a capacity of 10 ml, 1 ml of 1N NaOH was added, and the mixture was left to stand for 15 minutes. After 15 minutes, add 1 mL of 1N HCl to bring the solution up to the required concentration. Use a syringe filter to filter the solution, which will then be injected into the HPLC system.

Peroxide degradation

A volume of 1 ml sample stock solution was moved to a volumetric flask of 10 ml, add 1 ml of 30% hydrogen peroxide solution and make up to the mark with diluents. Filter the solution using syringe filter and injected into HPLC system.

Reduction degradation

Using a volumetric flask with a capacity of 10 ml, transfer 1 ml of sample stock solution and add 1 ml of 30% hydrogen peroxide solution, then dilute to the required concentration with diluents. Use a syringe filter to filter the solution, which will then be injected into the HPLC system.

Thermal degradation

During the 6-hour baking period, the sample solution was kept at 105°C. The resulting solution was injected into an high-performance liquid chromatography system.

Photolytic degradation

A weight of 100mg sample was exposed to sunlight for 6 hrs. and the exposed sample was analyzed. Prepare the sample solution by using this sample and inject into HPLC system.

Table 8. Forced degradation results

Stress Parameter (24 hrs)	% Degradation	
	Fenofibrate	Pitavastatin
Acid degradation (1N HCl)	12.4	13.4
Alkali degradation (1N NaOH)	13.2	13.1
Peroxide degradation (30% Peroxide)	13.8	14.5
Reduction degradation (30% sodium bi sulphate)	10.7	11.8
Thermal (sample, 70°C, 6 Hrs)	5.4	4.9
Photo (UV-Vis light- (200 W h/m^2) and fluorescent light (1.2 milliion lux-h)	4.9	3.5

4. Conclusion

In this study, a novel, quick, sensitive, and easy-to-use HPLC method was developed for the simultaneous estimation of Fenofibrate and Pitavastatin in API and pharmaceutical dosage types. Because there are no HPLC methods published, this approach is the most practical option. Shorter run time, low cost, and all the other characteristics are benefits. All the parameters were verified and were found to be within the acceptable range, including linearity, accuracy, specificity, robustness, and process precision. According to our research, the RSD values for all the parameters came in at less than 2%, showing that the procedure is accurate and that the results we found are consistent. Therefore, it's possible to use the current approach in QC laboratories for routine study and manufacturing Pitavastatin and Fenofibrate pharmaceuticals without having to separate the substances first.

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