Development of a New Validated Stability Indicating Method for Quantification of Fenofibric acid and Pitavastatin by Ultra Performance Liquid Chromatography

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Abstract

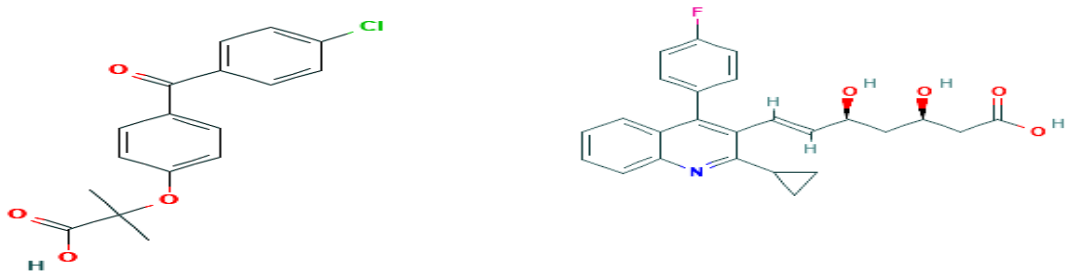
The main objective of the present work is to develop an efficient, unique, reliable Ultra performance liquid chromatographic method for the simultaneous quantificaiton of Fenofibric acid and Pitavastatin in bulk and Pharmaceutical formulations. The Chromatographic separation of the selected combination of drugs was performed on a Kinetex C_8 column (150mm x4.6mm, 2.6 µ) using an isocratic elution with a buffer containing 0.1% formic acid and acetonitrile at a ratio of 80:20 as a mobile phase with a flow rate of 0.5 mL/min at ambient temperature and wavelength at 266nm.The method produced reliable results at optimised chromatographic conditions. Analysis of honest linearity within a concentration range of 135-2025 µg/mL of fenofibric acid and 2-30 µg/mL of Pitavastatin with regression coefficients of 0.9992 and 0.9997 respectively was obtained. The retention time for fenofibric acid and pitavastatin were 1.778 min and 3.171 min respectively. LOD and LOQ were found to be 1.35 µg/mL, 0.02 µg/mL and 13.5 µg/mL, 0.2 µg/mL for fenofibric acid and pitavastatin respectively.The proposed method was validated in accordance with the guidelines of the International Council for Harmonization (ICH). All the obtained validation results of the proposed method were found to satisfactory and was succesfully applicable to the analysis of the bulk and the pharmaceutical formulations.

Key Words: *Fenofibric acid, Pitavastatin, Stability ,Validation, UPLC.*

Introductıon

Fenofibric acid chemically 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoic acid is a fibre class of drug used to treat abnormal levels of lipid in the blood and is less preferred to statin[1,2].It lowers lipid levels by activating peroxisome proliferator-activated receptor alpha (PPARα). PPARα activates lipoprotein lipase and reduces apoprotein CIII, which increases lipolysis and elimination of triglyceride-rich particles from plasma. As it does not appear to reduce the risk of heart disease[3,4] or death[5], it is recommended to be used together with dietary changes[6] by oral administration. Common side effects include problems with the liver[7,8], breathing problems[9], abdominal pain[10] muscle problems and nausea. Serious side effects may include toxic epidermal necrolysis[11], rhabdomyolysis[12], gall stone[13], blood clots and pancreatitis[14].

Pitavastatin (usually as calcium salt) chemically (E)-7- [2-cyclopropyl-4- (4-fluorophenyl) quinolin-3-yl]-3,5-dihydroxy- hept-6- enoic acid, is fully synthetic statin and inhibitor of HMG-CoA reductase. Pitavastatin is a more potent antihyperlipidemic agent compared to other statins. İt is odorless and looks like a white powder. It is hygroscopic in nature and very slightly unstable in sunlight. It is freely soluble in pyridine, chloroform, dilute HCl, DMSO and DMF. Like other statins, it is an inhibitor of HMG-CoA reductase, an enzyme that catalyses the first stage of the synthesis of cholesterol[15]. Common statin related side effects (head-ache, stomach upset, abnormal liver function tests and muscle cramps[16]) were similar to other statins. A study found that coenzyme Q10[17] was not reduced as much as with some other statins (though this is unlikely given the inherent chemistry of the HMG-CoA reductase pathway that inhibits all statin drugs[18,19]), hyperuricemia[20] or increased levels of serum uric acid with pitavastatin[21].



Fenofibric acid **Pitavastatin**

Figure 1. Chemical Structures of Fenofibric Acid and Pitavastatin

The literature survey till date found that there were methods available for individual estimation of the drugs but no analytical methods were found to be present for quantification of fenofibric acid and pitavastatin in combination for bulk and pharmaceutical formulation. The main objective of present work involves the production of a simple, rapid and accurate stability technique for estimating fenofibric acid and pitavastatin in bulk and pharmaceutical formulation by using UPLC.

Material and methods

Chemical and reagents

All HPLC grade solvents like acetonitrile, formic acid, water were purchased from Merck India Ltd, Worli, Mumbai, India. The required fenofibric acid, pitavastatin APIs as testimonal standards have been procured from Spectrum Pharma Research Solutions pvt Ltd, Hyderabad.

Equipment

The UPLC system (Agilent 1290 Infinity II LC system) consisting of a binary pump with PDA detector 2998 was used in the proposed work. Data processing was carried out with Empower - 2 software.

Preparation of Buffer

The buffer was prepared by transferring 1 mL of formic acid into 1000 mL volumetric flask which was dissolved in 500 mL of HPLC grade water. It was sonicated for 30 minutes and the volume was finally made upto 1000mL. This solution was filtered through 0.45 µ nylon syringe filter.

Preparation of mobile phase

The preparation of mobile phase was carried out by mixing Acetonitrile and 0.1% formic acid in proportion of 20:80 (% v/v) ratio. The resultant mixture was sonicated for 15 min and was filtered through 0.45 µ nylon syringe filter. The mobile phase was used as diluent in further procedures.

Preparation of standard solution

The Standard solution was prepared by transferring accurately weighed 1350 mg of fenofibric acid and 20 mg of pitavastatin working standards into a 100mL volumetric flask and diluted to volume with diluent. Further dilute 5 mL of above solution to 50 mL with diluent.The resultant concentration of solutions was 1350 µg/mL of fenofibric acid and 20 µg/mL of pitavastatin respectively.

Preparation of a sample solution

Accurately weighed 2220 mg of fenofibric acid and pitavastatin sample were transferred into 100 mL volumetric flask and add approximately 70 mL of diluent. It was Sonicated to dissolve and diluted with diluent. 5 mL of the above stock solution was further diluted to 50 mL and filtered through 0.45 µ nylon syringe filter. The final cocentration of solution obtained are equivalent to 1350 µg/mL (fenofibric acid) and 20 µg/mL (pitavastatin) respectively.

Chromatographic conditions

Chromatographic separation was performed in isocratic mode at ambient temperature using KinetexC_8 column (150mmx4.6mm, 2.6 µ). A mixture of 0.1% formic acid and acetonitrile in 80:20 %v/v at a flow of 0.5 mL/min was used as a movable phase. The volume of injection was 5µl and the duration of injection was 6 min.

Overlay Spectrum

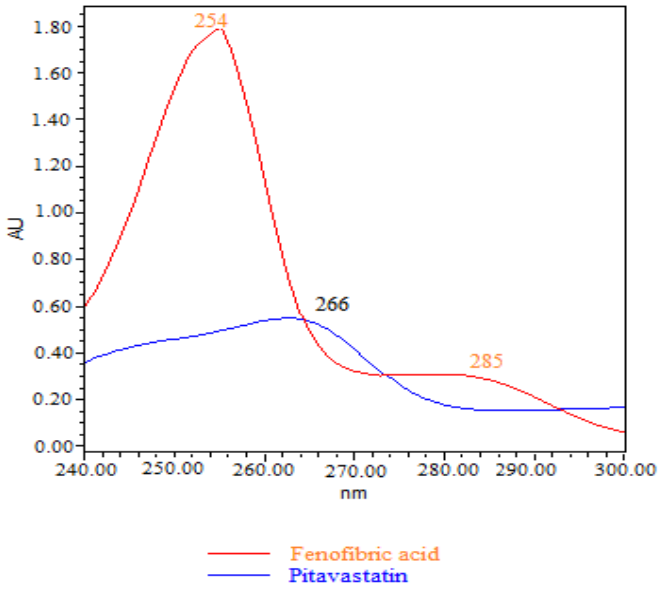


Figure 2. Overlay Spectrum of Fenofibric acid and Pitavastatin

Results and discussion

The current study was designed to develop a simple, accurate and rapid analytical UPLC method that can be used for the analysis of the assay method for the simultaneous estimation of fenofibric acid and pitavastatin in bulk and pharmaceutical dosage forms. Many trails were performed with various mobile phase compositions. The final optimized mobile phase is 0.1 percent formic acid and acetonitrile in 80:20 v/v ratio. The detection was carried out in several wavelengths in order to obtain sufficient sensitivity for the two APIs in a smaller proportion (fenofibric acid and pitavastatin). The drugs have exhibited good absorbance at a wavelength of 266 nm and it was considered for further study. The flow rate was 0.5 mL per minute. The retention time for fenofibric acid and pitavastatin were 1.778 min and 3.171 min respectively. The proposed method was validated in accordance with the guidelines of the ICH, with all results within the limit. The observation was made with a total run time of 6 min.

Validation:

Validation of analytical method is a process to establish performance characteristics of developed method which meets the requirement with intended analytical applications.

Table 1. Optimized chromatographic conditions

Parameter	Optimised condition
Stationary phase	Kinetex C_8 (150mmx4.6mm, 2.6 µ)
Mobile phase	Acetonitrile : 0.1% formic acid 20:80
Injection volume	5 µl
Flow rate	0.5 mL/min
Column temperature	Ambient

Wave length	266 nm
Run time	6 min
Retention time of fenofibric acid	1.778 min
Retention time of pitavastatin	3.171 min

System sutiability

System suitability parameters were performed to evaluate the system performance. The system sutiability was achieved by injecting a standard solution containing 1350 µg/mL of fenofibric acid and 20µg/mL of pitavastatin in six replicates. The results obtained for both the drugs indicate the system suitability parameter is within the limit.

Table 2. Results of system suitability

Parameter	Fenofibric acid	Pitavastatin
Theoretical plate count	2574	2510
Tailing factor	1.07	1.08
Resolution	-	7.6
Retention time (min)	1.778	3.171

Specificity

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix such as impurities, degradation products and matrix components.There was no interference from the blank at the retention time of both fenofibric acid and pitavastatin.

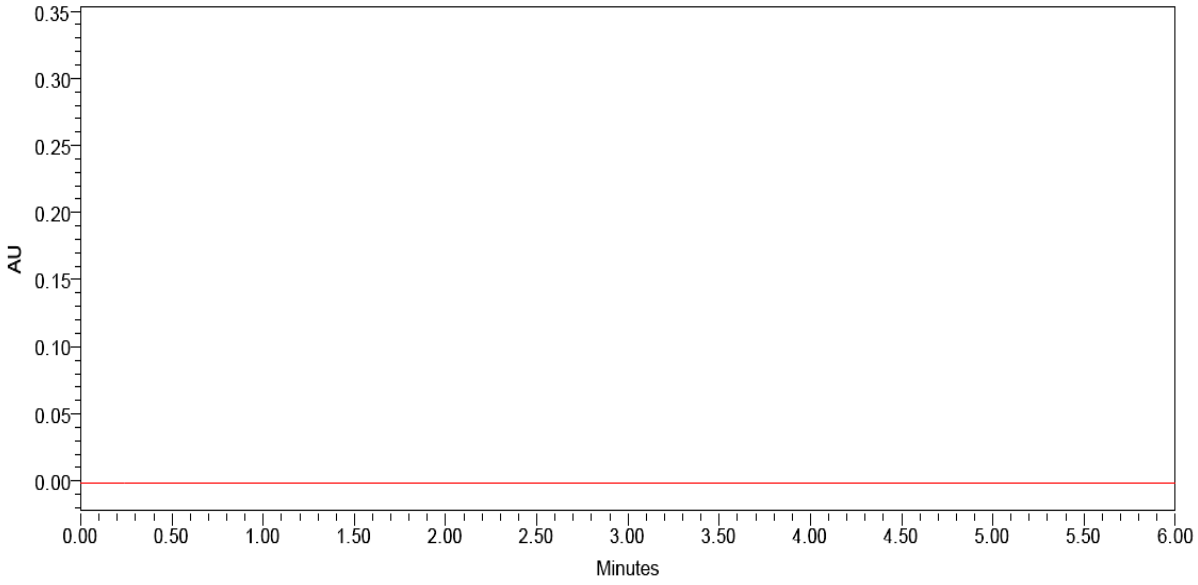


Figure 3. Chromatogram of Blank

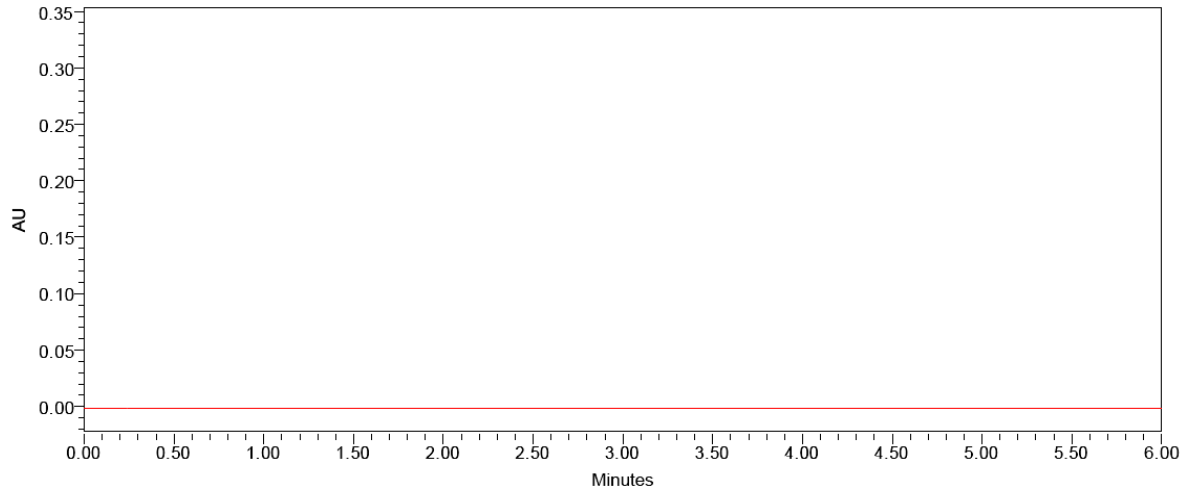


Figure 4. Chromatogram of Placebo

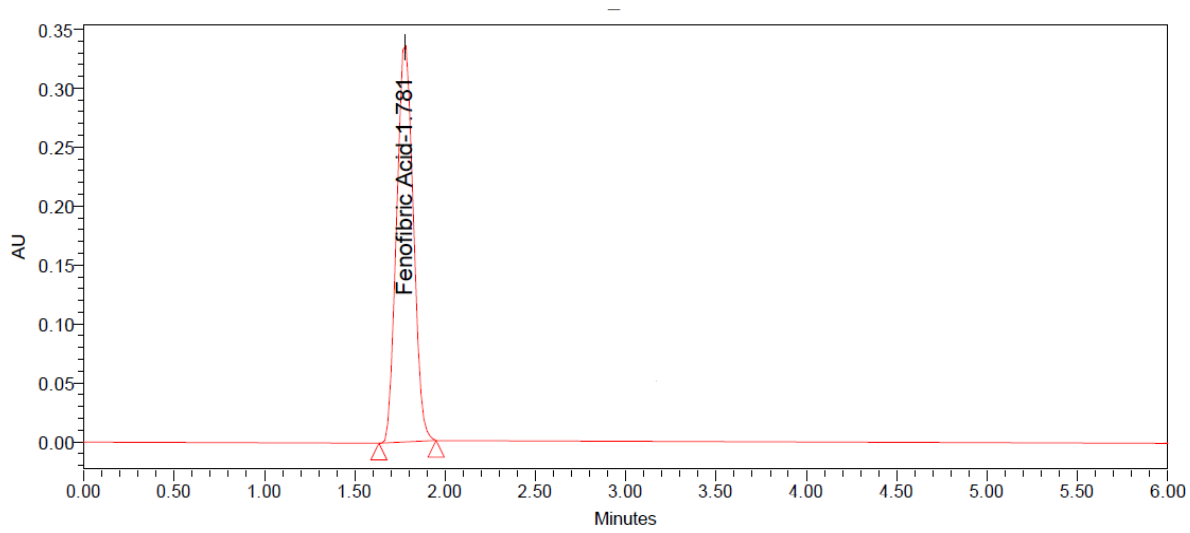


Figure 5. Chromatogram of Fenofibric acid

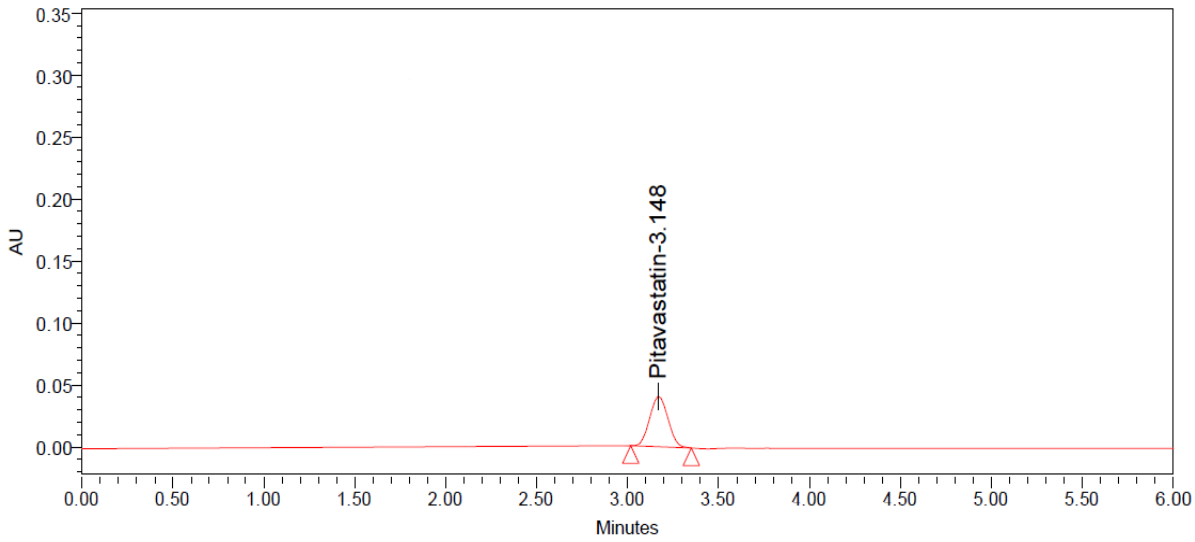


Figure 6. Chromatogram of Pitavastatin

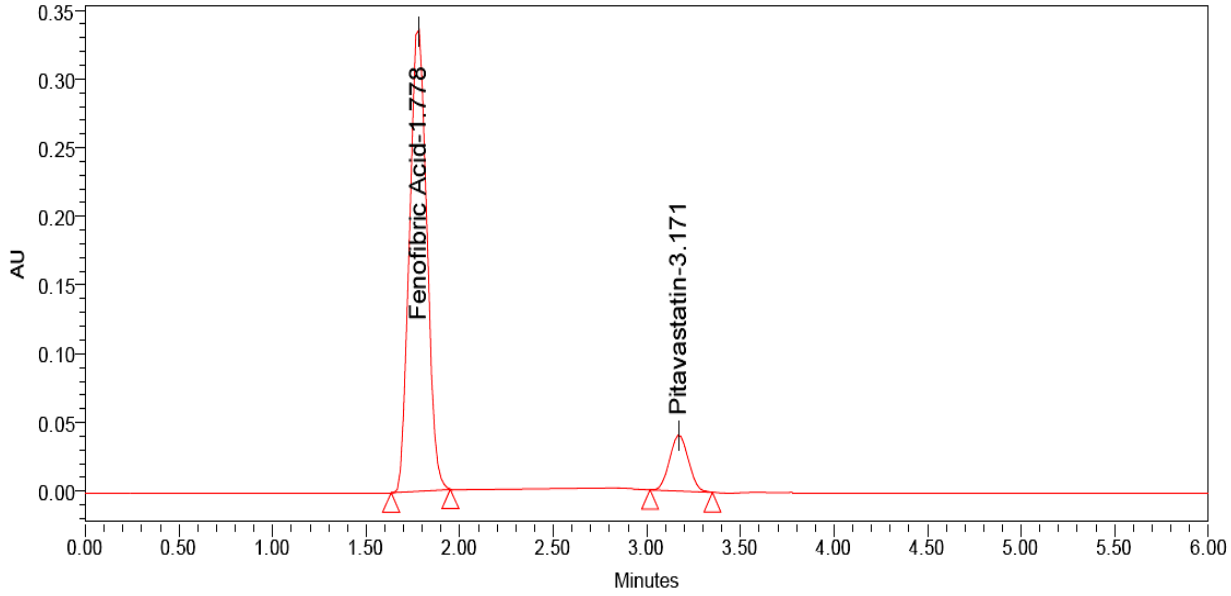


Figure 7. Chromatogram of Standard

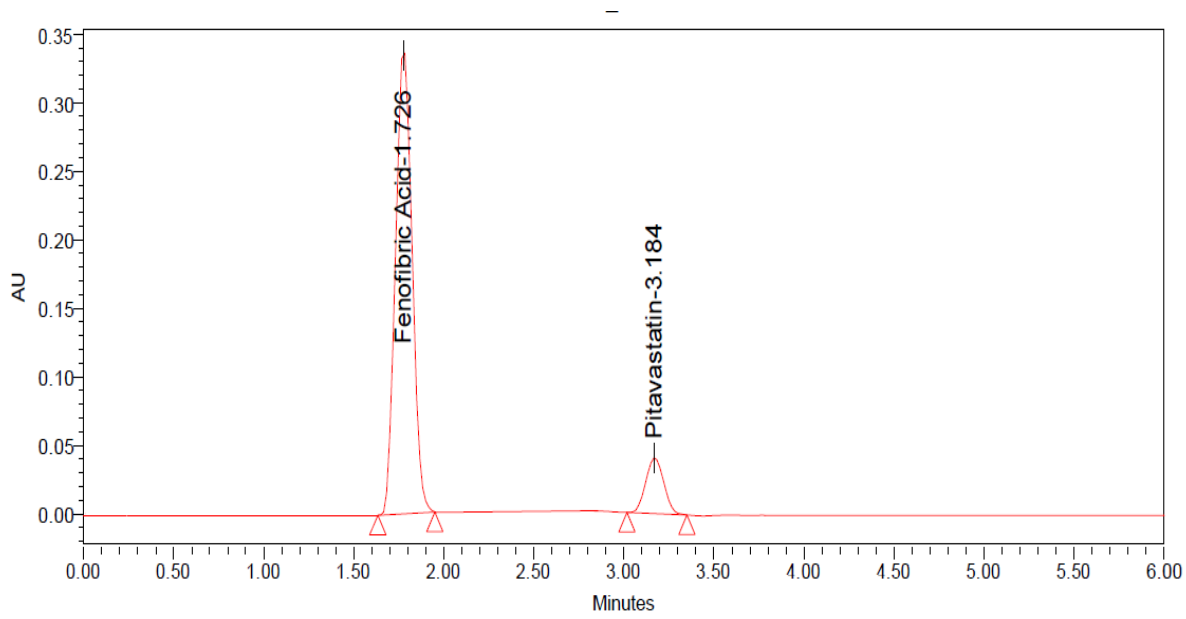


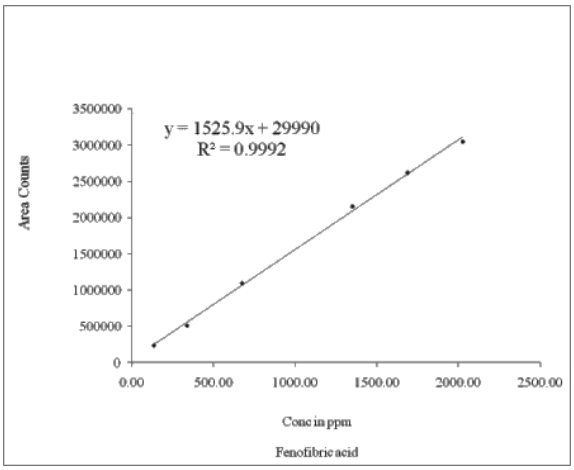
Figure 8. Chromatogram of Sample

Linearity

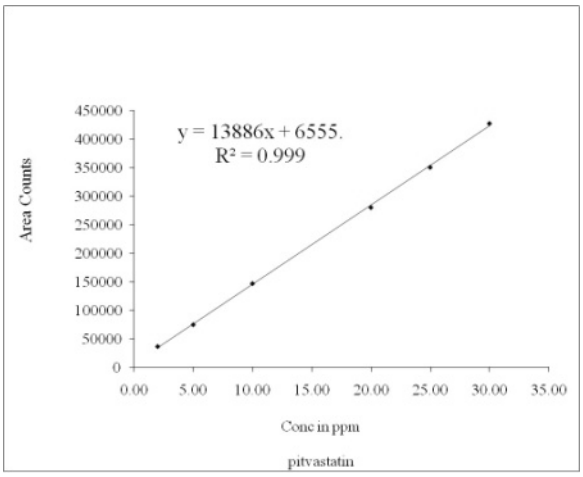
The linearity of an analytical method can be defined as the ability to obtain test results which are directly proportional to concentration in sample which was determined by injecting six replicates of each concentration and plotting the calibration curve of the peak response against the respective concentration. From this calibration curve, it was found that the curve was straight in the range of 135-2025 µg/mL of fenofibric acid and 2-30 µg/mL of pitavastatin. The regression equations for the calibration curves were Y=1525x+29989 (R^2-0.9992) and Y=13988x+4278 (R^2-0.9997) for fenofibric acid and pitavastatin respectively.

Table 3. Results of linearity

S No	Fenofibric acid		Pitavastatin	
	Concentratin µg/mL)	**Mean peak Area**	**Concentration (µg/mL)**	**Mean peak Area**
1	135	241110	2	36383
2	337	514159	5	74638
3	675	1097769	10	146882
4	1350	2158351	20	280360
5	1687	2622646	25	350920
6	2025	3051942	30	427679
Slope	1525.93		13988.18	
Intercept	29989.91		4278.44	
CC	0.99928		0.99976	



Linearity plot of Fenofibric acid



Linearity plot of Pitavastatin

Figure 8. Calibration plots of Fenofibric acid and Pitavastatin

Precision

The precision of an analytical method expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of this method was assessed in terms of intraday (repeatability) and intermediate variations. The intraday studies were determined by performing six repeated analyses of the sample solution of fenofibric acid (1350 µg/mL) and pitavastatin (20µg/mL)on the same day under the same experimental conditions. The intermediate precision of the method was carried out in the same laboratory by an analysis with

different analysts and different instruments. The method is highly accurate, as the percent RSD value was found to be < 2%. Good drug recovery was obtained at each concentration added, indicating that the method was accurate.

Table 4. Precision results of Fenofibric acid and Pitavastatin

S. No.	Area of Fenofibric acid		Area of Pitavastatin	
	Intraday precision	**Interday precision**	**Intraday precision**	**Interday precision**
1	2119571	2089123	285648	287654
2	2165840	2098154	281256	283216
3	2112354	2057854	281642	286241
4	2114343	2043215	288143	287548
5	2103214	2068974	285874	288452
6	2105321	2065820	286123	284543
Mean	2120274	2070523	284781	286276
SD	23066.805	20199.902	2732.372	2030.761
% RSD	1.09	0.98	0.96	0.71

Accuracy

The accuracy of an analytical method is the closeness of the test result obtained by that method to true value. It was achieved by calculating recovery experiments at three levels (50%, 100% and 150%). APIs containing 675, 1350, 2025 µg/mL of fenofibric acid and 10, 20, 30 µg/mL of pitavastatin were prepared. For each spike level, the sample solution was introduced three times and the test was performed as per the test method. The recovery results were close to 100 percent and the % RSD values were also less than 2 percent. Percent recovery, mean, relative standard deviations have been calculated. Recovery values have shown that the method is accurate within the desired range.

Table 5. Results of accuracy

Fenofibric acid					
Percentage of concentration (at the specification level)	**Area**	**Amount added (mg)**	**Amount found (mg)**	**% recovery**	**Mean recovery**
50	1087769	675	685.26	101.5	100.0
100	2169564	1350	1366.75	101.2	
150	3173654	2025	1999.03	98.7	
Pitavastatin					
50	142882	10	10.08	100.8	101.0
100	284021	20	20.03	100.2	
150	429614	30	30.29	101.0	

LOD and LOQ

Limit of detection and quantification of the minimum concentration at which the analyte is often reliably detected, quantified by the use of quality formulas. The LOD values of fenofibric acid and pitavastatin were 1.35, 0.02 µg/mL and their s/n values are 7, 3 and their LOQ values were found to be 13.5, 0.2 µg/mL and their s/n vlaues were 26, 23 respectively.

Robustness

The robustness is the capacity of method to remain unaffected by small but deliberate changes in chromatographic conditions. The robustness of the chromatographic method was determined by the variable flow rate and the mobile phase composition. The RSD percentage was found to be within the acceptable limit.

Table 6. Results of robustness

Parameter	% RSD of fenofibric acid	% RSD of pitavastatin
Flow (0.8 mL/min)	1.21	0.42
Flow (1.2 mL/min)	0.64	1.58
Org phase (18:82)	0.43	0.44
Org phase (22:78)	1.58	0.42

Forced Degradation

Stock solution preparation

1350 mg of fenofibric acid and 20 mg of pitavastatin are accurately weighed and transferred to a 100 mL volumetric flask, 70 mL of diluents were added and sonicated to dissolve for 30 minutes and make up for the diluents mark.

Acid degradation

1mL of the sample stock solution was transferred to a 10mL volumetric flask, 1 mL of 1N HCl was added and kept for 15 minutes. After 15 minutes, 1mL of 1N NaOH was added and made up the diluents mark.

Alkali degradation

1mL of the sample stock solution was transferred to a 10mL volumetric flask, 1 mL of 0.1N NaOH was added and kept for 15 minutes. After 15 minutes 1mL of the 1N HCl was added and made up the mark with diluents.

Peroxide degradation

1mL of the sample stock solution was transferred to a 10mL volumetric flask, 0.3 mL 30 percent hydrogen peroxide was added and made up to the mark with diluents.

Reduction degradation

1 mL of the sample stock solution was transferred to a 10 mL volumetric flask, 1 mL of 30 percent sodium bisulphate solution was added and made up to the mark with diluents.

Thermal degradation

The sample solution was set in an oven at 105º for 6 hours. The resultant solution was injected into HPLC.

Hydrolysis degradation

1 mL of sample stock was transferred to a 10 mL volumetric flask, 1mL of water was added and made up to the mark with diluents.

All the above final solutions were injected into system and % degradation was observed. Forced degradation studies were conducted on the basis of ICH requirements including acid, base, oxidation, reduciton, thermal and hydrolysis degradation. It is evident from chromatograms that the selected drugs were stable under the applied stress conditions although the degraded peaks were observed.

Table 7. Results of forced degradation

Degradation condition	Fenofibric acid				
	Area	**Percentage degradation**	**Purity angle**	**Purity Threshhold**	**Pass/fail**
Control	2146196	-0.1	0.338	10.826	Pass
Acid degradation	2056528	4.1	0.364	10.871	Pass
Alkali degradation	2066358	3.6	0.357	10.871	Pass
Peroxide degradation	2055874	4.1	1.174	10.328	Pass
Reduction degradation	2066574	3.6	0.36	10.876	Pass
Thermal degradation	2056237	4.1	0.369	10.875	Pass
Hydrolysis degradation	2050574	4.4	0.365	10.873	Pass
Pitavastatin					
	Area	**Percentage degradation**	**Purity angle**	**Purity Threshhold**	**Pass/fail**
Control	284417	-0.1	4.185	10.719	Pass
Acid degradation	275641	3	4.174	10.732	Pass
Alkali degradation	273645	3.7	4.177	10.73	Pass

Peroxide degradation	271456	4.4	2.546	10.605	Pass
Reduction degradation	274825	3.3	4.178	10.731	Pass
Thermal degradation	271453	4.4	4.168	10.742	Pass
Hydrolysis degradation	274631	3.3	4.166	10.741	Pass

Conclusion

In this study a novel, rapid, economical, sensitive and readily available UPLC method was developed for the simultaneous estimation of fenofibric acid and pitavastatin in bulk and the marketed formulation. This was the first reported method as per the literature survey till date. This method exhibits shorter runtime, low price accessibility, sensitivity, reliability and reproducibility. These characteristics are significant when it is essential to evaluate a large number of samples. Validation of all parameters such as linearity, accuracy, specificity, robustness, method precision were carried out and found to be within the appropriate limits. RSD values of all the required parameters were found to be less than 2%, which means that the method is valid and the results derived are in fair agreement. The prefered method could therefore easily be applied for the normal investigation and for the pharmaceutical formulations of fenofibric acid and pitavastatin in quality control laboratories without any preliminary separation.

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Conflicts of ınterest

The authors declare that there was no conflicts of interest.

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