

STABILITY INDICATIVE AND COST EFFECTIVE ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF FENOFIBRIC ACID AND PITAVASTATIN BY USING RP-HPLC

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ABSTRACT

Objective: The current investigation was pointed at developing and progressively validating novel, simple, responsive and stable RP-HPLC method for the measurement of active pharmaceutical ingredients of Fenofibric acid and Pitavastatin.

Methods: A simple, selective, validated and well-defined stability that shows gradient RP-HPLC methodology for the quantitative determination of Fenofibric acid and Pitavastatin. The chromatographic strategy utilized X-bridge phenyl column of dimensions 250x4.6 mm, 5 micron, using isocratic elution with a mobile phase of acetonitrile and 0.1 percent formic acid (60:40). A flow rate of 1 ml/min and a detector wavelength of 242 nm utilizing the PDA detector were given in the instrumental settings. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines.

Results: LOD and LOQ for the two active ingredients were established with respect to test concentration. The calibration charts plotted were linear with a regression coefficient of $R^2 > 0.999$, means the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness were determined as a part of method validation and the results were found to be within the acceptable range.

Conclusion: The proposed method to be fast, simple, feasible and affordable in assay condition. During stability tests, it can be used for routine analysis of the selected drugs.

Keywords: Fenofibric acid, Pitavastatin, RP-HPLC, Development, Validation

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INTRODUCTION

The active form of fenofibrate, a synthetic phenoxy-isobutyric acid derivate having antihyperlipidemic [1] action, is fenofibric acid. Fenofibric acid is a lipid-lowering [2] drug used to treat severe hypertriglyceridemia [3, 4], primary hyperlipidemia, and mixed dyslipidemia. It operates by lowering high-density lipoprotein [5, 6] cholesterol, total cholesterol, triglycerides [7], and apolipoprotein B [8, 9] while raising low-density lipoprotein cholesterol. The prodrug, [fenofibrate], and other conjugated derivatives of fenofibric acid, such as choline fenofibrate, have been created for enhanced solubility, gastrointestinal [10] absorption, and bioavailability [11], as well as more convenient administration due to its high hydrophilicity and poor absorption profile.

Pitavastatin (usually as a calcium salt) is a member of the blood cholesterol [12] lowering medication class of statins [13, 14]. It inhibits HMG-CoA reductase [15], the enzyme that catalyses the first step in cholesterol production, like other statins. Pitavastatin, like the other statins, is used to treat hypercholesterolemia [16, 17] (high cholesterol) and to prevent cardiovascular disease [18, 19]. The side effects of common statins (headaches, stomach upset, abnormal liver function tests, and muscle cramps [20]) were similar to those of other statins. Pitavastatin, on the other hand, appears to cause fewer muscular adverse effects than some lipid-soluble statins, owing to its water-soluble nature (as is pravastatin, for example). Coenzyme Q10 [21] was not lowered as much as other statins (albeit this is unexpected given the underlying chemistry of the HMG-CoA reductase pathway, which all statin medicines inhibit) [22, 23]. Pitavastatin, in contrast to other statins, has been shown to ameliorate insulin resistance [24] in humans, as measured by the homeostatic model assessment (HOMA-IR) method [25]. Pitavastatin has been linked to hyperuricemia [26] and higher levels of blood uric acid [27]. The aim of the study is to separate the pharma ingredients Fenofibric and Pitavastatin by using RP-HPLC.

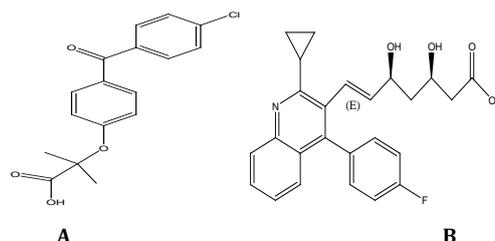


Fig. 1: Structure of (A) Fenofibric acid and (B) Pitavastatin

MATERIALS AND METHODS

Chemicals

Acetonitrile, HPLC-grade formic acid, water were purchased from Merck India Ltd, Mumbai, India. APIs of Fenofibric acid, Pitavastatin standards were procured from Glenmark, Mumbai.

The Instrumentation

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

Method optimization

To optimize the chromatographic conditions, different ratios of phosphate buffer and the acetonitrile in the mobile phase with isocratic and gradient mode was tested. However, the mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention times. Finally, 0.1% formic acid buffer and acetonitrile with isocratic elution was selected because it results in a greater response of active pharmacy ingredients. During the optimization of the method various stationary phases such as C₈, C₁₈ and amino, phenyl columns were

tested. From these trials the peak shapes were relatively good with X-bridge phenyl column of 250 x 4.6 mm, 5 μ with a PDA detector. The mobile phase flow rate has been done at 242 nm in order to obtain enough sensitivity. By using the above conditions, we get retention times of Fenofibric acid and Pitavastatin were about 2.7 min and 7.3 min with a tailing factor of 1.02 and 1.34. The number of theoretical plates for Fenofibric acid and Pitavastatin were 5216, 7421, which indicate the column's successful output the % RSD for six replicate injections was around 0.08% and 0.16%, the proposed approach suggests that it is extremely precise. According to ICH guidelines, the method established was validated.

Till today there are no HPLC methods were reported in the literature, but only few methods are developed in individual analysis of Fenofibric acid and Pitavastatin. Hence we developed method for the simultaneous quantification of Fenofibric acid and Pitavastatin. The developed HPLC method was utilized for the estimation of the combined drugs by *in vitro* method. Different extractions were tried using acetonitrile, methanol, and dimethylformamide.

Validation procedure

The analytical parameters such as system suitability, precision, specificity, accuracy, linearity, robustness, LOD, LOQ, forced degradation and stability were validated according to ICH Q2 (R1) guidelines [28, 29].

Preparation of buffer

1 ml of formic acid is dissolved in 1 lt of HPLC grade water and filter through 0.45 μ filter paper.

Chromatographic conditions

The HPLC analysis was performed on a reverse-phase HPLC system with isocratic elution mode using a mobile phase of acetonitrile and 0.1% formic acid and X-bridge phenyl (250x4.6 mm, 5 μ) column with a flow rate of 1 ml/min.

Diluent

Mobile phase was used as a diluent.

Preparation of the standard stock solution

For standard stock solution preparation, add 70 ml of diluents to 175 mg of Fenofibric acid and 10 mg of Pitavastatin taken in a 100 ml volumetric flask and sonicate for 10 min to fully dissolve the contents and then make up to the mark with diluent.

Preparation of Standard solution

1 ml of solution is drawn from the above normal stock solution into a 10 ml volumetric flask and diluted up to the level.

RESULTS AND DISCUSSION

The main analytical challenge during the development of a new method was to separate active Pharma ingredients. In order to provide a good performance, the chromatographic conditions were optimized.

System suitability

In System, suitability injecting standard solution and reported USP tailing and plate count values are tabulated in table 1.

Table 1: Results of system suitability

| System suitability parameter | Acceptance criteria | Drug name | |
|------------------------------|---------------------|-----------------|--------------|
| | | Fenofibric acid | Pitavastatin |
| USP Plate Count | NLT 2000 | 5216 | 7421 |
| USP Tailing | NMT 2.0 | 1.02 | 1.34 |
| USP Resolution | NLT 2.0 | - | 20.43 |
| % RSD | NMT 2.0 | 0.08 | 0.16 |

Specificity

In this test method placebo, standard and standard solutions were analyzed individually to examine the interference. The below fig. shows that the active ingredients were well separated from blank and their excipients and there was no interference of placebo with the principal peak. Hence the method is specific.

Linearity

The area of the linearity peak versus different concentrations has been evaluated for Fenofibric acid, Pitavastatin, as 10, 25, 50, 100, 125, 150 percent, respectively. Linearity was performed in the range of 17.5-262.5 μ g/ml of Fenofibric acid and 1-15 μ g/ml of Pitavastatin. The correlation coefficients achieved greater than 0.999 for all.

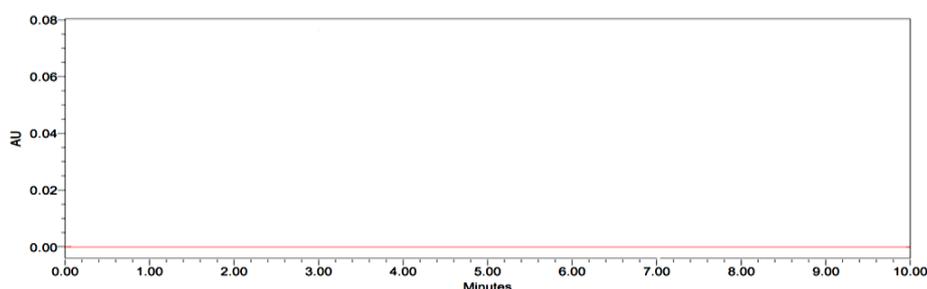


Fig. 3: Chromatogram of blank

Table 2: Linearity of fenofibric acid and pitavastatin

| S. No. | Conc μ g/ml | Fenofibric acid area count | Conc. μ g/ml | Pitavastatin area count |
|-------------|-----------------|----------------------------|------------------|-------------------------|
| 1 | 17.50 | 336520 | 1.00 | 57846 |
| 2 | 43.75 | 886321 | 2.50 | 174632 |
| 3 | 87.50 | 1702453 | 5.00 | 295684 |
| 4 | 175.00 | 3265942 | 10.00 | 563245 |
| 5 | 218.75 | 4056897 | 12.50 | 722013 |
| 6 | 262.50 | 4832016 | 15.00 | 859564 |
| Correl coef | | 0.99982 | | 0.99933 |
| Slope | | 18347.61 | | 56618.31 |
| intercept | | 44332.33 | | 9791.70 |

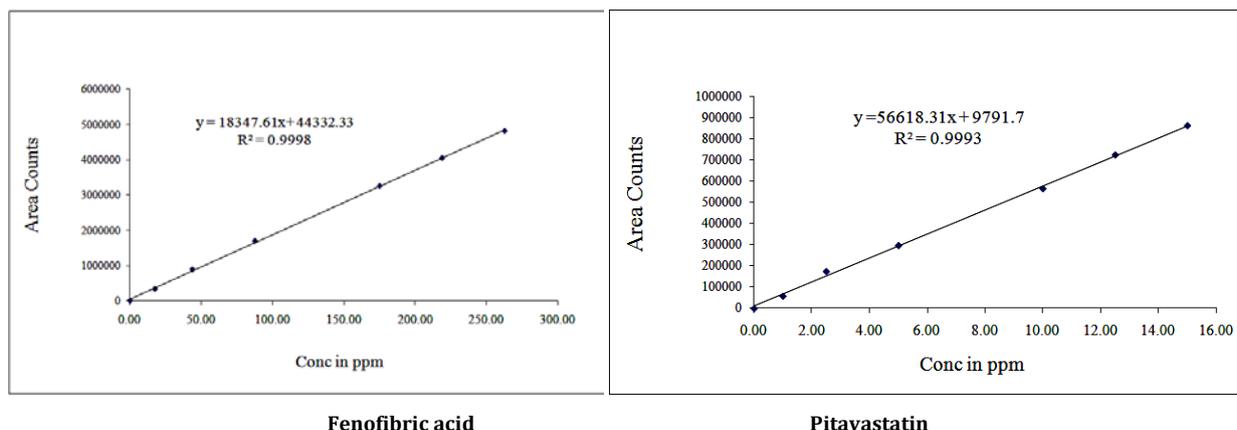


Fig. 4: Calibration plots of (A) Fenofibric acid (B) Pitavastatin

Accuracy

In this method, Accuracy was conducted in triplicate by analyzing active pharma ingredient standard solution at three kinds of concentration levels of 50, 100 and 150% of each at a specified limit.

For all impurities, percentage recoveries were measured and found to be within the limit. The accuracy and reliability of the developed method were established. The percentage recovery values were found to be in the range of 99.97-100.66% for Fenofibric acid and 99.73-99.94% for Pitavastatin. The results are given in table 3.

Table 3: Results of accuracy

| S. No. | % Level | Fenofibric acid % recovery | Pitavastatin % recovery |
|--------|---------|----------------------------|-------------------------|
| 1 | 50 | 99.97 | 99.73 |
| 2 | 100 | 100.54 | 99.94 |
| 3 | 150 | 100.66 | 99.88 |
| mean | | 100.39 | 99.85 |
| SD | | 0.369 | 0.108 |

Mean+SD (n=3)

Precision

In method precision study, prepare six different standard solutions in the concentration of Fenofibric acid (175 ppm) and Pitavastatin (10 ppm) are injected into HPLC system. Fenofibric acid %assay found to be in the range of 99.74-100.63 and Pitavastatin %assay found to be in the range of 99.48-100.85. These results are given below table 4.

Intraday precision

Six replicates of a standard solution containing Fenofibric acid (175µg/ml) and Pitavastatin (10µg/ml) were analysed on the same day. Peak areas were calculated, which were used to calculate mean, SD and %RSD values.

Intermediate precision

Six replicates of the standard solution were studied by various researchers, and on separate days different instruments were tested. The peak regions used to determine to mean percent RSD values have been calculated. The results are given in the following table.

Inter-day precision

Six replicates of a standard solution containing Fenofibric acid (175µg/ml) and Pitavastatin (10µg/ml) were analysed on a different day. Peak areas were calculated, which were used to calculate mean, SD and %RSD values. The present method was found to be precise as the RSD values were less than 2% and also the percentage assay values were close to be 100%. The results are given in table 5.

Table 4: Intraday precision results of fenofibric acid and pitavastatin

| Fenofibric acid | | | | Pitavastatin | | |
|-----------------|---------------|-------------|---------------|---------------|-------------|---------------|
| S. No. | Conc. (µg/ml) | Area counts | % assay as is | Conc. (µg/ml) | Area counts | % Assay as is |
| 1 | 175 | 3265021 | 100.49 | 10 | 567482 | 100.12 |
| 2 | | 3245786 | 101.05 | | 563201 | 99.48 |
| 3 | | 3285647 | 100.63 | | 564178 | 100.85 |
| 4 | | 3263023 | 100.24 | | 563296 | 100.16 |
| 5 | | 3248798 | 99.74 | | 562388 | 100.45 |
| 6 | | 3247859 | 99.96 | | 564527 | 100.28 |
| % RSD | 0.468 | | | 0.317 | | |
| mean | | | 100.35 | | | 100.3575 |
| SD | | | 0.474 | | | 0.119252 |

Mean+SD (n=6)

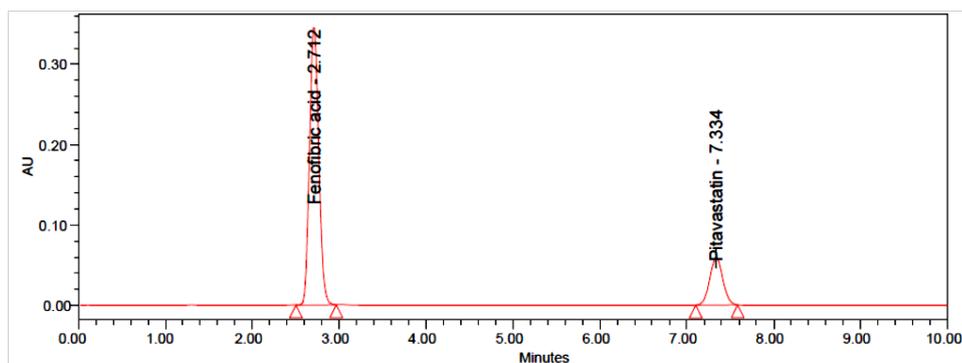


Fig. 5: Chromatogram of method precision

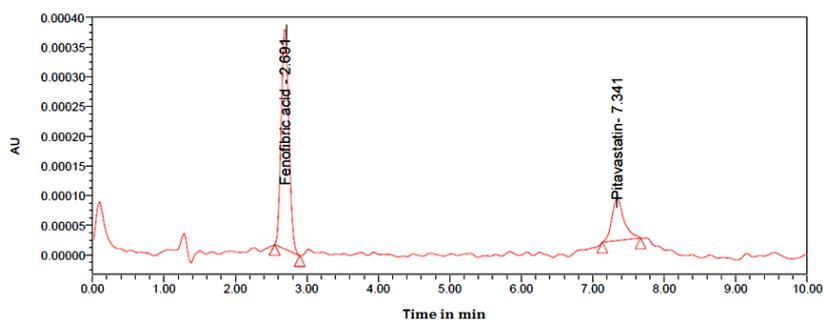
Table 5: Inter-day outcomes of the accuracy of fenofibric acid and pitavastatin

| Fenofibric acid | | | Pitavastatin | | | |
|-----------------|---------------|-------------|---------------|---------------|------------|---------------|
| S. No. | Conc. (µg/ml) | Area counts | % Assay as is | Conc. (µg/ml) | Area count | % Assay as is |
| 1 | | 3263635 | 100.54 | 10 | 569386 | 100.44 |
| 2 | 175 | 3245187 | 100.27 | | 568245 | 98.63 |
| 3 | | 3249587 | 100.63 | | 563214 | 99.04 |
| 4 | | 3206315 | 101.15 | | 567421 | 98.75 |
| 5 | | 3285632 | 100.39 | | 568239 | 99.64 |
| 6 | | 3276942 | 99.68 | | 565210 | 99.68 |
| %RSD | 0.868 | | | 0.406 | | |
| Mean | 100.44 | | | 99.36 | | |
| SD | 0.481 | | | 0.686 | | |

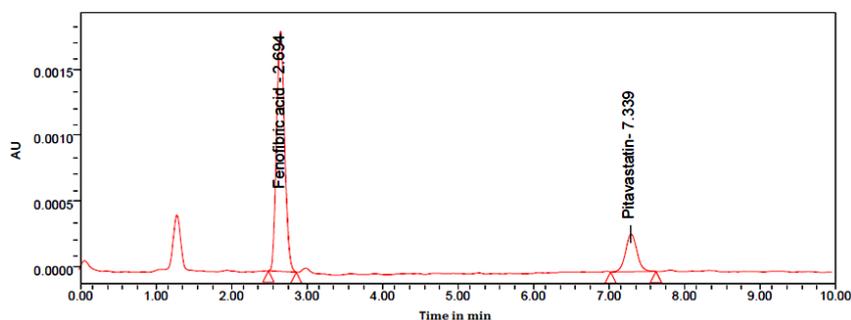
Mean+SD (n=6)

Table 6: LOD and LOQ for fenofibric acid and pitavastatin

| Fenofibric acid | | | Pitavastatin | | | | |
|-----------------|-----|---------------|--------------|---------------|-----|---------------|-----|
| LOD | | LOQ | | LOD | | LOQ | |
| Concentration | s/n | Concentration | s/n | concentration | s/n | Concentration | s/n |
| 0.219µg/ml | 7 | 0.722µg/ml | 28 | 0.013µg/ml | 3 | 0.041µg/ml | 23 |



A



B

Fig. 6: Chromatogram of (A) LOD and (B) LOQ

LOD and LOQ

The LOD concentrations for Fenofibric acid are 0.219 µg/ml and s/n values is 7 and Pitavastatin 0.013 µg/ml and s/n value 3. The LOQ concentration for Fenofibric acid 0.722 µg/ml and their s/n values are 28 and Pitavastatin 0.041 µg/ml and s/n value is 23. The method is validated as per the US FDA guidelines [30].

Robustness

The conditions of the experiment were designed to test the robustness of the established system intentionally altered, such as flow rate, mobile phase in organic percentage in all these varied conditions. Robustness results for Fenofibric acid and Pitavastatin found to be within the limit and results are tabulated in table 7.

Table 7: Robustness data of fenofibric acid and pitavastatin

| Parameter name | % RSD | |
|-------------------------|-----------------|--------------|
| | Fenofibric acid | Pitavastatin |
| Flow minus (0.8 ml/min) | 0.74 | 0.88 |
| Flow plus (1.2 ml/min) | 0.29 | 0.54 |
| Organic minus (-10%) | 1.56 | 0.91 |
| Organic plus (+10%) | 0.83 | 0.74 |

Table 8: Stability results of fenofibric acid and pitavastatin

| Stability | Fenofibric acid | | Pitavastatin | |
|-----------|-----------------|----------------|--------------|----------------|
| | Purity | % of deviation | Purity | % of deviation |
| Initial | 99.99 | 0.01 | 99.99 | 0.01 |
| 6 H | 99.75 | 0.22 | 99.61 | 0.37 |
| 12 H | 99.56 | 0.47 | 99.42 | 0.49 |
| 18 H | 99.31 | 0.63 | 99.27 | 0.74 |
| 24 H | 99.11 | 0.84 | 98.97 | 0.97 |

Stability

The standard and standard solution was kept at room temperature and at 2-8 °C up to 24 h. Then these solutions were pumped into the device and calculate the % of deviation from initial to 24 h [31]. There was no significant deviation observed and confirmed that the solutions were stable up to 24 h percentage of the assay was not quite 2%. There is no effect in storage conditions for Fenofibric acid and Pitavastatin drugs. The results are given below table 8.

Degradation studies

The Pitavastatin and Fenofibric acid standard was subjected into various forced degradation conditions to effect partial degradation of the drug. Studies of forced degradation have been carried out to find out that the method is suitable for products of degradation [32, 33]. In addition, the studies provide details about the conditions during which the drug is unstable, in order that the measures are often taken during formulation to avoid potential instabilities [34].

Acid degradation

Acid degradation was done by using 1N HCl and 15.42% of Fenofibric acid and 14.76% of Pitavastatin degradation was observed.

Alkali degradation

Alkali degradation was done at 1N NaOH and 14.96% of Fenofibric acid and 14.22% of Pitavastatin degradation was observed.

Peroxide degradation

Peroxide degradation was performed with 30% hydrogen peroxide and 13.25% Fenofibric acid, 13.96% of Pitavastatin degradation was observed.

Reduction degradation

Reduction degradation was performed with 30% sodium bi sulphate solution, 12.47% Fenofibric acid and 12.54% Pitavastatin degradation was observed.

Thermal degradation

In thermal degradation, the standard was degraded to 12.11% of Fenofibric acid and 11.37% of Pitavastatin.

Degradation of hydrolysis

In hydrolysis degradation, the standard was degraded to 11.63% of Fenofibric acid and 11.59% of Pitavastatin.

All degradation results are tabulated in table 9.

Table 9: Forced degradation results of fenofibric acid and pitavastatin

| Degradation condition | Fenofibric acid | | Pitavastatin | |
|------------------------|-----------------|-------|--------------|-------|
| | % Assay | % Deg | % Assay | % Deg |
| Acid degradation | 84.58 | 15.42 | 85.24 | 14.76 |
| Alkali degradation | 85.04 | 14.96 | 85.78 | 14.22 |
| Peroxide degradation | 86.75 | 13.25 | 86.04 | 13.96 |
| Reduction degradation | 87.53 | 12.47 | 87.46 | 12.54 |
| Thermal degradation | 87.89 | 12.11 | 88.63 | 11.37 |
| Hydrolysis degradation | 88.37 | 11.63 | 88.41 | 11.59 |

CONCLUSION

We present in this article simple, selective, validated and well-defined stability that shows gradient RP-HPLC methodology for the quantitative determination of Fenofibric acid and Pitavastatin. All the products of degradation formed during the stress conditions and the related active pharma ingredients are well separated and peaks

were well resolved from each other and separate with an appropriate retention time, indicating that the proposed method to be fast, simple, feasible and affordable in assay condition.

Therefore the developed method during stability tests, it can be used for routine analysis of production standards and to verify the quality of drug standards during stability studies.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

Declared none

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