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Development and Validation of Fast, Simple RP-HPLC Method for Simultaneous Estimation of Atorvastatin and Fenofibrate in Tablet Dosage Form

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Abstract:
Background*: A simple, specific, linear, precise and accurate reverse phase liquid chromatographic method was developed for the simultaneous determination of Atorvastatin calcium and Fenofibrate in tablet dosage forms. The chromatographic separation was performed using Hypersil BDS C8 Column (125 mm x 4.0 mm, 5 µm particle size). Mobile phase composed of buffer and acetonitrile (40:60 v/v) was selected and a flow rate of 1.6 ml/minute is monitored with injection volume of 20 µl. Detection was carried out at 276 nm. The method was validated as per ICH guidelines. The retention time for Atorvastatin and Fenofibrate are observed as 1.43 and 4.37 minutes respectively. Linearity range was observed in concentration of 4.8 – 14.3 µg/ml for Atorvastatin and 81.9 – 245.8 µg/ml for Fenofibrate. The percentage recoveries of Atorvastatin and Fenofibrate are 99% and 99% respectively. The correlation coefficients for both the components are close to 1. The proposed method was validated and successfully applied to the estimation of Atorvastatin calcium and Fenofibrate in tablet dosage forms.*

Key Word*: Atorvastatin, Fenofibrate, RP-HPLC, Validation.*

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I. Introduction

Atorvastatin is chemically named as (3*R*, 5*R*)-7-(2-[4-fluorophenyl]-3-phenyl-4-[phenylcarbamoyl]-5-propan-2-ylpyrrol-1-yl)-3,5-dihydroxyheptanoic acid (Fig. 1). It is a member of the drug class known as statins, which is HMG-COA reductase inhibiter act as anti- hyper lipidemic drug clinically effective drug in the treatment of Hyper cholestrimia. It is soluble in methanol, ethanol, and acetonitrile. Practically insoluble in water. [1],[2].

Fenofibrate is chemically named as propan2-yl2-{4-[(4- chlorophennyl)carbonyl] phenoxy}-2methylpropionate is a widely used as Anti-cholesrtimic agent as *ppar* receptor inhibiter [2],[6]. Literature survey reveals that few spectrophotometric methods and high performance liquid chromatography (HPLC) methods have been reported for the estimation of Atorvastatin & Fenofibrate.

The aim of this study is to develop a simple, precise and accurate reversed-phase HPLC (RP-HPLC) method for the estimation of Atorvastatin and Fenofibrate in pharmaceutical dosage forms as per ICH guidelines [3]. The validation procedure followed the guidelines of USP 30 [4].

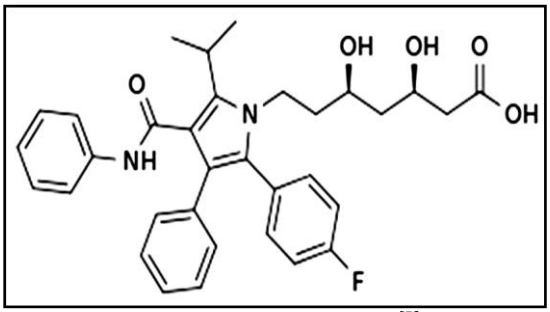

Figure 1 : Atorvastatin [5]

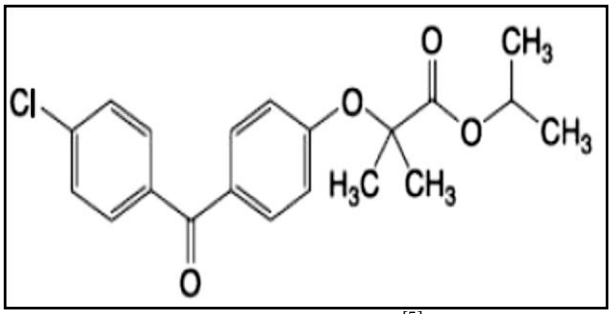

Figure 2 : Fenofibrate [5]

II. Material And Method Development

Instrumental and analytical conditions:

The HPLC analysis was carried with Dionex Ultimate 3000 HPLC system with PDA detector and auto sampler integrated with Chromeleon software Version 7.2.10

The column used is Hypersil BDS C8 column (125 mm x 4.0 mm, 5 µm particle size) and detection was performed at 276 nm. The injection volume of sample was 20 µl and the run time was 5 minutes. An isocratic mobile phase consisted of buffer and acetonitrile (40:60 v/v). The mobile phase was filtered through 0.45 µm membrane filter and degassed before use.

Reagents and chemicals

Atorvastatin & Fenofibrate were taken from commercial source and tablets were obtained from Medley Pharmaceutical Limited. HPLC grade acetonitrile was obtained from Merck (India) Ltd. All other chemicals used were AR grade.

Preparation of buffer solution

Take 4.0 g of Potassium Orthophosphate in 1000 ml of water. Add to it 0.5 ml of Orthophosphoric acid

Preparation of mobile phase

Mix buffer and acetonitrile in the ration of 40:60 v/v. Mobile phase is degassed before use.

Preparation of standard solution

Solution 1: Weigh & transfer accurately about 25.0 mg of Atorvastatin working standard in to 50 ml volumetric flask. Add to it 30 ml of mobile phase and sonicate for 5 minutes. Dilute up to the mark with Mobile phase & mix well.

Solution 2: Weigh & transfer accurately about 40.0 mg of Fenofibrate working standard in to 25 ml volumetric flask. Add to it 15 ml of mobile phase and sonicate for 5 minutes. Dilute up to the mark with Mobile phase & mix well.

Pipette out 2.0 ml of solution 1 and 10.0 ml of solution 2 in 100 ml volumetric flask. Dilute up to the mark with mobile phase. Mix well & inject.

Preparation of sample solution

Take 20 tablets and crush. Mix uniformly and take average weight of powder in 100 ml volumetric flask. Add to it 70 ml of mobile phase, sonicate for 15 minutes, cool to room temperature. Further dilute 5 ml of above solution to 50 ml with mobile phase. Mix well & inject.

Method development

Various mobile phase combinations were tried initially to separate Atorvastatin and Fenofibrate on C8 column. In order to achieve acceptable peak shapes and perform the separation on a suitable run time various buffer systems are also tried systematically. Mobile phase composed of buffer and acetonitrile (40:60 v/v) indicated that the resolution between Atorvastatin and Fenofibrate increased. Therefore buffer and acetonitrile (40:60 v/v) at a flow rate of 1.6 ml/minute was selected as optimized mobile phase. Hypersil BDS C8 column (125 mm x 4.0 mm, 5 µm particle size) was used as the stationary phase to improve resolution. To analyze both drugs, detection was tried at various wavelengths but 276 nm was selected as the detection wavelength as both the drugs showed maximum absorption. The retention time was found to be 1.43 and 4.37 minutes for Atorvastatin and Fenofibrate respectively. The chromatogram obtained was shown in Figure (3). The system suitability parameters were shown in Table (1).

Table 1: System Suitability Parameters

Parameter	Atorvastatin	Fenofibrate
Retention time	1.423	4.367
Tailing factor	1.16	1.04
% RSD	0.04	0.04

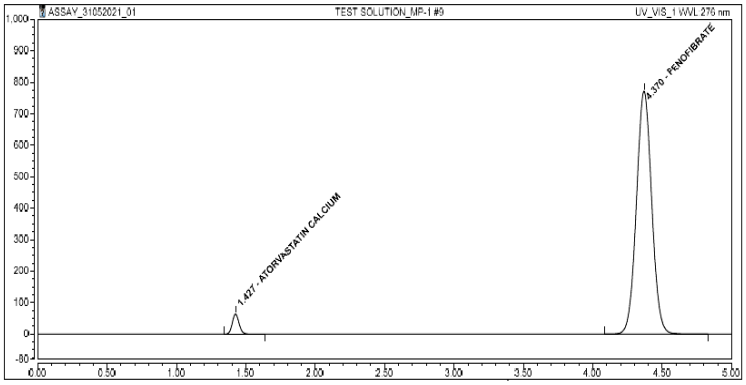

Figure 3: Representative chromatogram of test solution

III. Material Validation

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The above method was validated according to ICH guidelines to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method. They were tested using the optimized chromatographic conditions and instruments. [3], [5]

Specificity

Spectral purities of atorvastatin and Fenofibrate chromatographic peaks were evaluated for the interference of the tablet excipients, degradation components or due to the presence of impurities as per the methodology. In the work, a solution containing a mixture of the tablet excipients were prepared using the sample preparation procedure to evaluate possible interfering peaks. [7]

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity of detector response for Atorvastatin and Fenofibrate was established by analyzing serial dilutions of a stock solution of the working standard. [5] Five concentrations such as 4.8, 7.6, 9.5, 11.4 & 14.3 µg/ml for Atorvastatin and 81.9, 131.1, 163.8, 196.6 & 245.8 µg/ml Fenofibrate are prepared as per table (2) & (3) and analyzed. Correlation coefficient & %Y-axis intercept should be within the limit.

Table 2: Linearity Concentration Levels of Atorvastatin

% level	Volume of stock solution	Diluted to (ml)	Final concentration in ppm
50%	0.5 ml	50	4.8
80%	0.8 ml	50	7.6
100%	1.0 ml	50	9.5
120%	1.2 ml	50	11.4
150%	1.5 ml	50	14.3

Table 3: Linearity Concentration Levels of Fenofibrate

% level	Volume of stock solution	Diluted to (ml)	Final concentration in ppm
50%	2.5 ml	50	81.9
80%	4.0 ml	50	131.1
100%	5.0 ml	50	163.8
120%	6.0 ml	50	196.6
150%	7.5 ml	50	245.8

Accuracy

The accuracy of the method was determined by recovery experiments known concentrations of working standard was added to the fixed concentration of the pre-analyzed Tablet sample. Percent recovery was calculated by comparing the area with pre-analyzed sample. For both the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 50%, 100%, 150% level and the percentage recovery was calculated. Data from the linearity was considered for accuracy. Refer table (2) & (3).

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under prescribed conditions. Repeatability of the method was checked by injecting replicate injections of 9.5 µg/ml for Atorvastatin & 163.8 µg/ml for Fenofibrate of the solution for 6 times. The mean area and % relative standard deviation (RSD) was calculated. % RSD should be ≤ 2 %.

Intermediate precision

The intermediate precision of the assay method is established by comparison of two independent repeatability experiments on 2 different days. The data of the 1st day is taken from the analysis of "Repeatability". The second set of experiments is performed by a different analyst and HPLC system as well. The standard deviation, relative standard deviation and mean value difference is calculated from the results obtained on each day.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was observed that the variations like flow rate & mobile phase composition etc.

IV. Result & Discussion

Atorvastatin and Fenofibrate showed maximum absorbance at 276 nm. The proposed method for standard addition simultaneous estimation of both the drugs was validated as per the ICH guidelines.

Specificity:

By comparing the chromatograms of blank solution, placebo solution, reference solution & test solution it is observed that there is no interference of any peaks at the retention time of Atorvastatin as well as Fenofibrate. The retention time of the main peaks (Atorvastatin & Fenofibrate) in the chromatogram obtained with the reference solution & test solution are matching. This confirmed the specificity of the method.

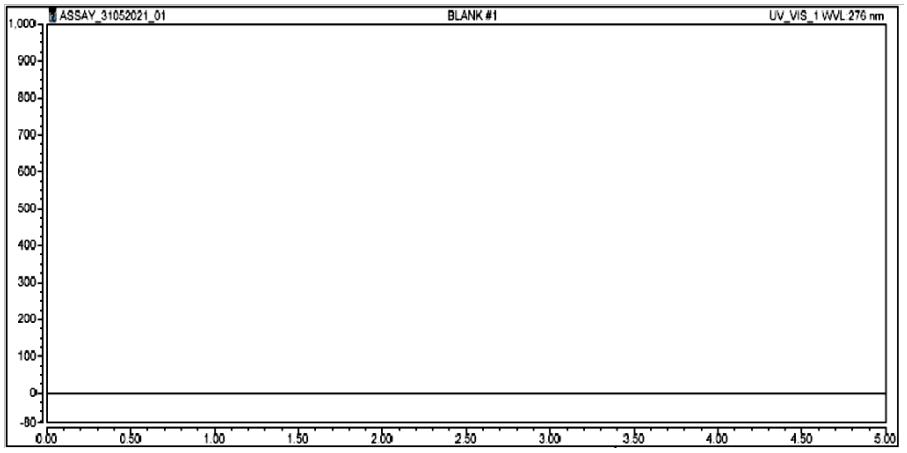

Figure 4: Chromatogram of blank solution

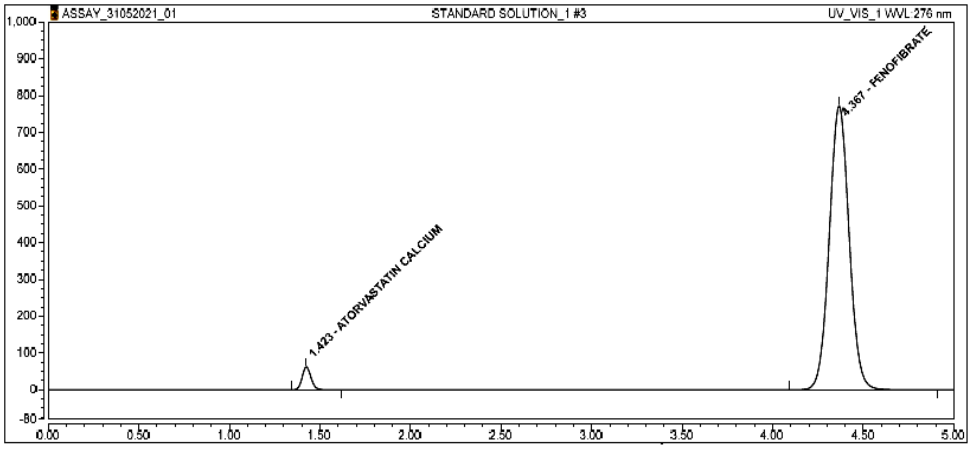

Figure 5: Chromatogram of standard solution

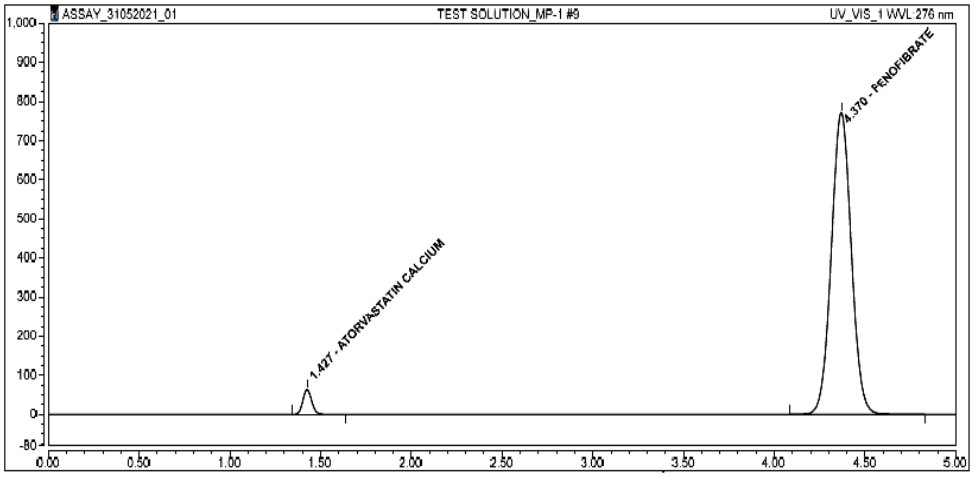

Figure 6: Chromatogram of sample solution

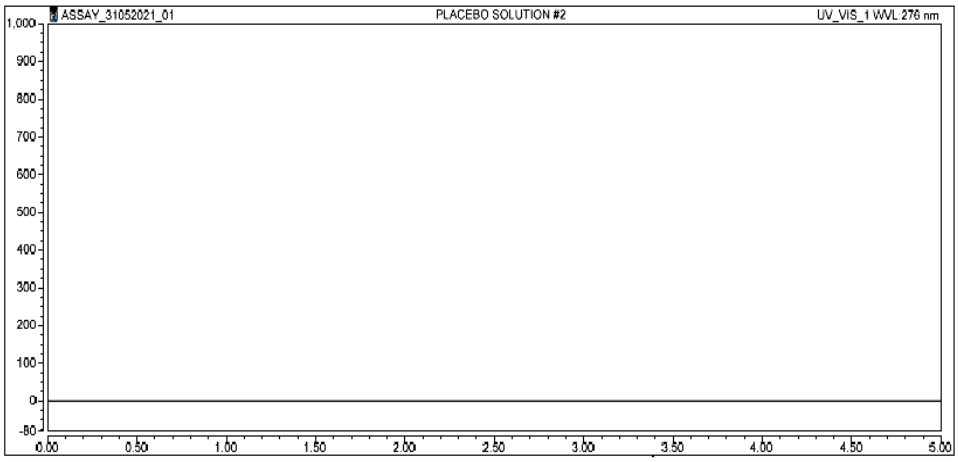

Figure 7: Chromatogram of placebo solution

Linearity

Five concentrations such as 4.8, 7.6, 9.5, 11.4 & 14.3 µg/ml for Atorvastatin and 81.9, 131.1, 163.8, 196.6 & 245.8 µg/ml Fenofibrate were prepared and the linearity graph was plotted using concentration verses peak area and shown in Figures (8) and (9). A linear relationship was obtained between peak areas and quantity analyzed in the range of 50 to 150% (4.8 – 14.3 µg/ml for Atorvastatin and 81.9 – 245.8 µg/ml for Fenofibrate).

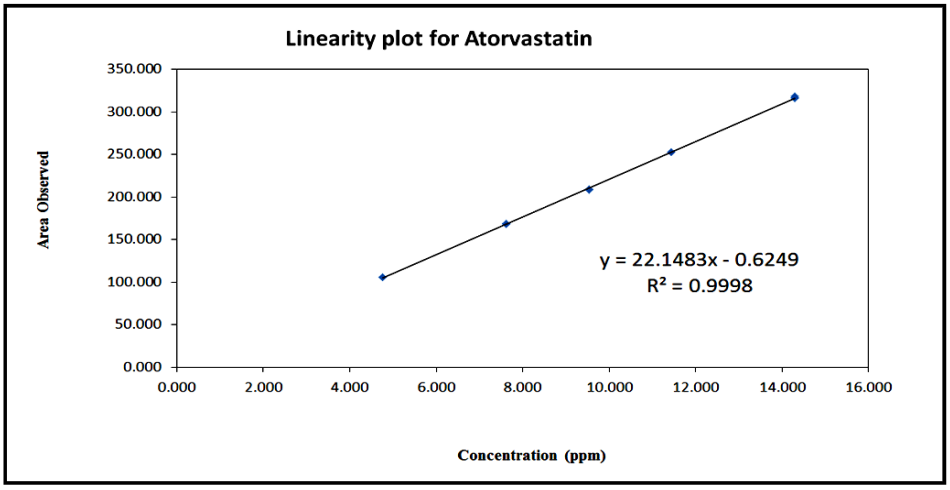

Figure 8: Linearity plot for Atorvastatin

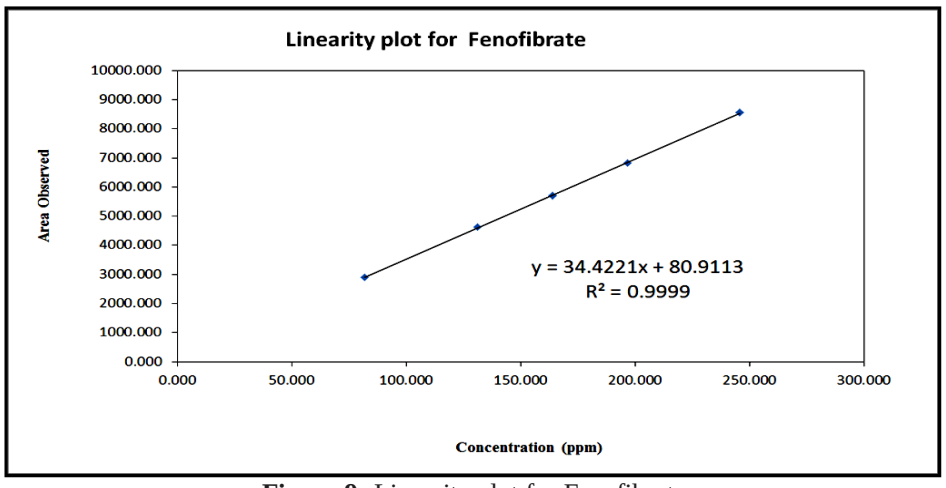

Figure 9: Linearity plot for Fenofibrate

A plot of Residuals against concentration for Aatorvastatin & Fenofibrate is show in figure (10) & (11) respectively.

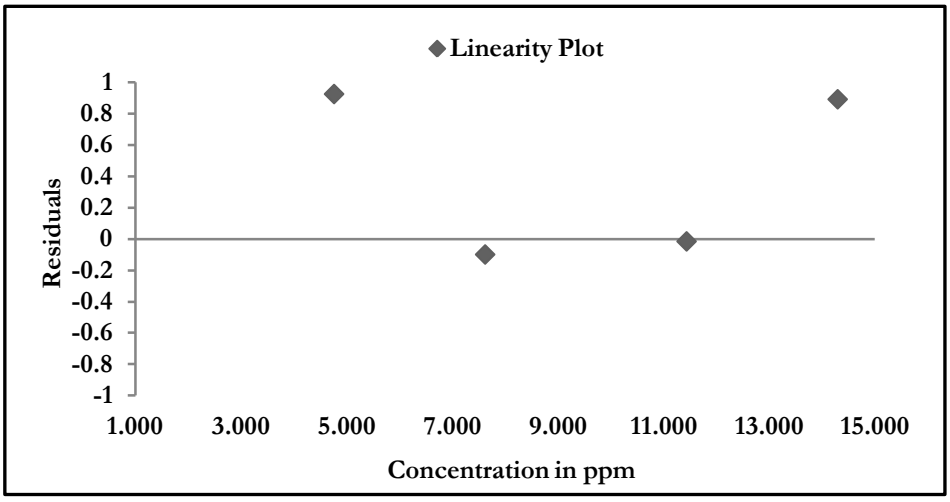

Figure 10: Plot of Residuals to test concentration for Atorvastatin

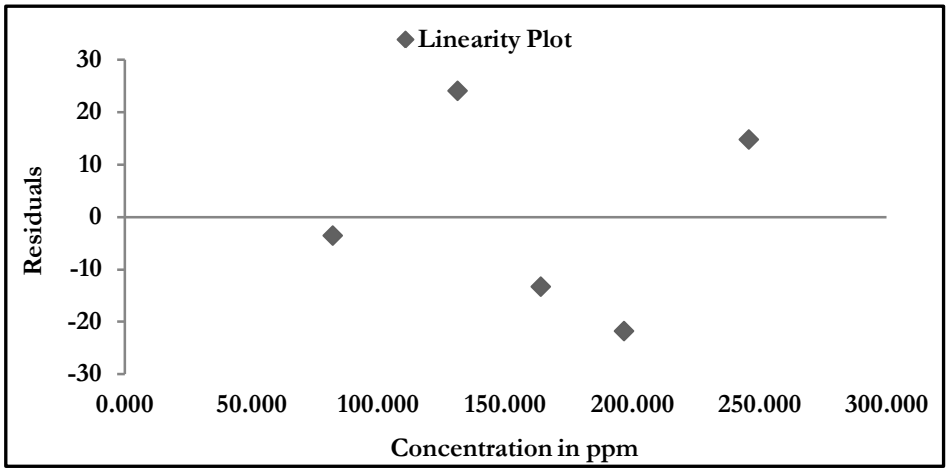

Figure 11: Plot of Residuals to test concentration for Fenofibrate

Observation table:

Table 4: For Atorvastatin

Parameter for Linearity	Values	Acceptance Criteria
Correlation coefficient R	1.000	$\geq$ 0.999
%Y – axis intercept	- 0.30	$\leq \pm$ 3 %
Slope of regression line	22.1	To be reported
Residual sum of squares	16.3	To be reported

Table 5: For Fenofibrate

Parameter for Linearity	Values	Acceptance Criteria
Correlation coefficient R	1.000	$\geq$ 0.999
%Y – axis intercept	1.41	$\leq \pm$ 3 %
Slope of regression line	34.4	To be reported
Residual sum of squares	9599.5	To be reported

The method is considered to be linear in the range on 4.8 – 14.3 µg/ml for atorvastatin and 81.9 – 245.8 µg/ml for Fenofibrate as Correlation coefficient & %Y-axis intercept should be within the limit.

Accuracy

The percentage recoveries of atorvastatin and Fenofibrate were 99% and 99% respectively, which shows the accuracy of the method. Refer table (6) for recovery at different concentration levels. The recovery values between prescribed limit of 98-102 % shows that method is free from interference of excipients present in formulation.

Table 6: Recovery at Different Concentration Levels

Accuracy level	% recovery of Atorvastatin	% recovery of Fenofibrate
50%	98	99
100%	99	99
150%	100	99
Means recovery	**99**	**99**

Precision

The exactness of the method as defined by precision and method is considered to be precised as since the relative standard deviation from 6 determinations is well within the acceptance limit. Refer table (7).

Table 7: Method Precision

Sample No.	% Assay of Atorvastatin	% Assay of Fenofibrate
Sample 01	101.9	101.5
Sample 02	101.0	100.7
Sample 03	101.3	101.0
Sample 04	101.8	101.4
Sample 05	101.9	101.5
Sample 05	101.4	101.0
Mean	**101.5**	**101.2**
STD Dev	**0.38**	**0.36**
% RSD	**0.4**	**0.4**

Intermediate Precision

The intermediate precision of the assay method is established by comparison of two independent repeatability experiments on 2 different days. Refer table (8).

Table 8: Intermediate Precision

Sample No.	% Assay of Atorvastatin	% Assay of Fenofibrate
Sample 01	100.4	100.5
Sample 02	100.4	100.5
Sample 03	101.2	101.1
Sample 04	101.0	100.7
Sample 05	102.4	102.2
Sample 06	100.0	100.2
Mean	**100.9**	**100.8**
STD Dev	**0.85**	**0.73**
% RSD	**0.8**	**0.7**

Robustness

Method is found to be robust as system suitability criteria is achieved for all the robustness parameters tested. Deliberate change in parameter does not have any significant effect on the method performance, which demonstrated that the developed RP-HPLC method is robust. The results were shown in Table (9) & (10).

Table 9: Robustness Result For Atorvastatin

Parameter	System suitability		% Assay
	% RSD	Tailing factor	
Flow rate			
1.4 ml/min	0.1	1.16	99.8
1.6 ml/min	0.1	1.10	99.8
Column oven temperature			
30ºC	0.1	1.11	99.6
40ºC	0.1	1.10	99.3

Table 10: Robustness Result for Fenofibrate

Parameter	System suitability		% Assay
	% RSD	Tailing factor	
Flow rate			
1.4 ml/min	0.1	1.06	99.9
1.6 ml/min	0.1	1.06	100.0
Column oven temperature			
30ºC	0.1	1.06	99.6
40ºC	0.1	1.05	99.3

V. Conclusion

In this present work a new simple, selective, linear, precise, accurate and robust HPLC method was developed and validated for the estimation of atorvastatin and Fenofibrate in pharmaceutical tablet dosage form in accordance with the ICH guidelines. This method gives good resolution between both the compounds with a short analysis time. Thus, this method can be useful for the routine analysis of atorvastatin and Fenofibrate combined in pharmaceutical tablet dosage form.

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