Simultaneous Method Development and Validation of Atorvastatin and Fenofibrate by RP-HPLC Method in Pharmaceutical Formulation

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ABSTRACT - A simple RP-HPLC method was developed and validated for estimation of Atorvastatin and Fenofibrate in tablet dosage form. Determination of the drugs were carried out on Inertsil ODS (2,250 x 4.6mm; packed with particle size of 5µ) column with a mixture of pH 2.5 phosphate buffer and acetonitrile in the ratio 18:82%v/v as mobile phase at a flow rate of 1.0ml/min at ambient temperature. Detection was monitored at 260 nm. The retention time was 3.5 and 7.06 min for Atorvastatin and Fenofibrate respectively. The validation parameters of the developed method such as specificity, linearity, precision, accuracy, stability and robustness were evaluated as per ICH guidelines. Linearity of the method was achieved over the concentration range of 5.003 – 60.03 µg/ mL for Atorvastatin and 144.225-480.750µg/ mL for Fenofibrate. The LOD and LOQ values were found to be 1.005 and 3.04 µg/ mL for Atorvastatin whereas 5.47 and 16.60 µg/ mL for Fenofibrate respectively. The developed method can be adopted for simultaneous determination of Atorvastatin and Fenofibrate in tablet dosage forms.

Keywords: Atorvastatin, Fenofibrate, ICH guidelines, Development, Validation

INTRODUCTION

Atorvastatin is chemically (3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid with the molecular formula $C_{33}H_{35}FN_2O_5$ and molecular weight of 558.65g/mol. It is an antihyperlipedemic agent belonging to the class of statins.[1] It is known act by inhibiting Hydroxymethylglutaryl-CoA (HMG-CoA) reductase enzyme which is involved in the synthesis of cholesterol and hence used as lipid lowering agent.[2,3] It is primarily used in patients with high risk of coronary heart disease (CHD) and also to reduce myocardial infarction and other cardio vascular disorder.[4-6]

Fenofibrate is chemically propan-2-yl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoatewith the molecular formula $C_{20}H_{21}ClO_4$ and molecular weight of 360.834g/mol.[7] It is another antihyperlipedemic agent belonging to the class of fibrates.[8] It acts by activating peroxisome proliferator activated receptor alpha (PPARalpha) which further activates lipoprotein lipase activity by reducing the production of apoprotein C-III which is a lipoprotein lipase inhibitor.[9,10] This results in increased lipolysis and hence used to reduce total cholesterol, LDL cholesterol thereby increasing HDL cholesterol levels.[11]

Literature shows several validation methods to determine Atorvastatin and Fenofibrate which include LC-ESI-MS method[12], HPLC,[13-15]HPTLC method,[16] UPLC method,[17] UV spectroscopic method[18-20] and few RP-HPLC methods[21-23]. However, all the methods reported were either individually validated or with other drugs. The main objective of this study was to develop a method to determine Atorvastatin and Fenofibrate simultaneously which can be simple, stable, accurate, robust and economical as per ICH guidelines.[24]

MATERIAL AND METHODS

The drugs Atorvastatin andFenofibrate were obtained as a gift samples from Hetero labs, Hyderabad. Acetonitrile used was of HPLC grade and water used was of Milli-Q grade. Atorvastatin and Fenofibrate tablets**(FIBATOR®S, Sun Pharma)**were purchased from local nearby pharmacy.

Instrument and chromatographic conditions

Determination and validation of the drugs were performed on HPLC (Shimadzu) with dual reciprocating plunger design (LC 20AT) with autosampler and diode array detector. Analytical column used was Inertsil ODS (2,250 x4.6mm; packed with particle size of 5µ) and the injection volume was set to 20µl. The system was equipped with EMPOWER software. Mobile phases used was the mixture of pH 2.5 phosphate buffer and acetonitrile in the ratio 18:82%v/v at a flow rate of 1.0 ml/min with the run time set at 12 minutes. The mobile phase was filtered through 0.45µm membrane filter. Temperature of the column was ambient with the UV detection at 220nm and the diluent used was the mixture of phosphate buffer of pH 2.5 and acetonitrile in the ratio 20:80%v/v.

Preparation standard stock solution

10mg of Atorvastatin and 145mg of Fenofibrate were dissolved in 60ml of diluent, sonicated for 5 minutes and were then diluted with diluent upto 100.0ml in a 100.0ml volumetric flask.

Preparation of working standard solution

5ml of Atorvastatin and 10ml of Fenofibratestandard stock solution were taken on 50ml volumetric flask and sonicated for 5 minutes with intermittent shaking and then diluted up to the mark with diluents.

Preparation of sample solution

About 20 tablets ere weighed and powdered and then the powder containing equivalent amount of 10mg of Atorvastatin, 145mg of Fenofibrate was taken in a 100ml volumetric flask. About 70ml of the diluent was added and shaken on a rotary flask for 20mins followed by addition of further 30ml of diluent. It was then sonicatedfor 20mins to remove any dissolved gases present. From this, 5ml of sample solution was pipetted into a 25ml volumetric flask and diluted up to the mark with diluents and filtered through 0.45µ membrane filter to obtain clear solution.

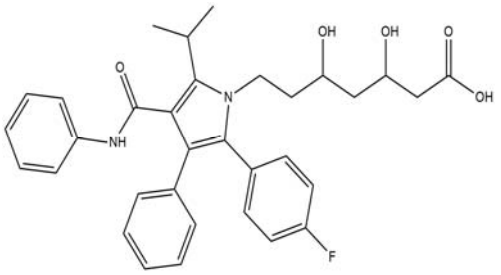

Figure 1: Structure of Atorvastatin

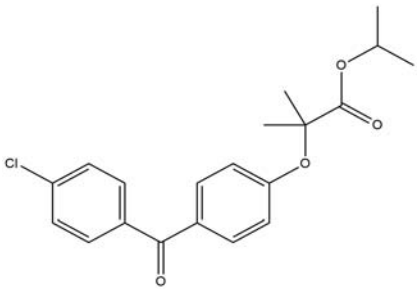

Figure 2: Structure of Fenofibrate

RESULTS AND DISCUSSION

Method optimization

Optimization of the method was achieved through evaluation of different solvent mixtures and columns. Mobile phase in the ratio 18:82%v/v of pH 2.5 phosphate buffer and acetonitrile on the column Inertsil ODS (2,250 x 4.6mm; packed with particle size of 5µ) produced two peaks which were well resolved with good peak shape and symmetry, and the retention time for Atorvastatin, and Fenofibrate were 3.5 and 7.06 respectively. The chromatogram of the optimized trial is shown in the figure3.

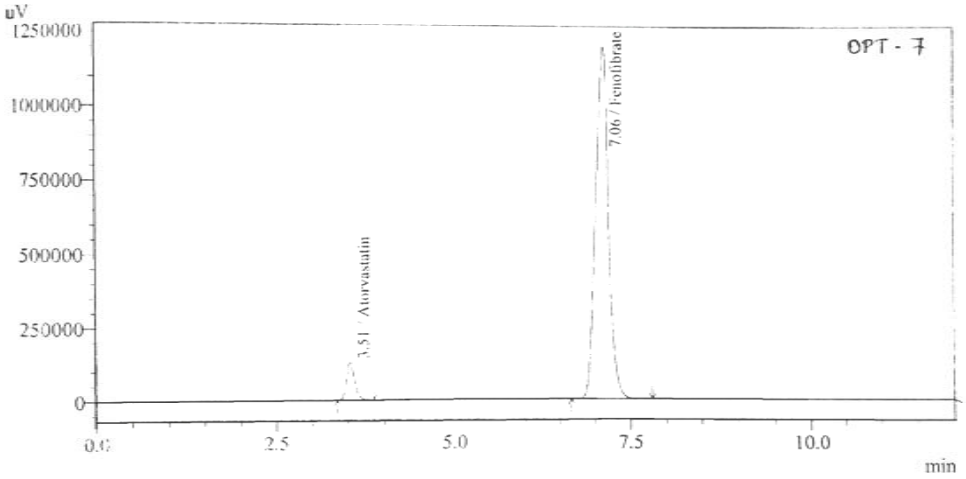

Figure 3: Typical chromatogram of optimized trial

Specificity and System suitability

A study was conducted to establish the interference of excipients. Samples were prepared by taking placebo equivalent to about the weight in portion of test preparation as per the test method. The chromatogram of placebo did not show any extra peakswhich indicate that the excipients used in formulation do not interfere in the assay.

Forced degradation studies

Atorvastatin and Fenofibrate drug substance and product were prepared as per test method and were stressed under various degradation conditions such as acidic, basic, peroxide, thermal, photostability and humidity. The samples were then injected into the chromatographic system. The peaks obtained were homogenous and has no co-eluting peaks at the retention times of both the drugs which determines that the peak purity passes for each stress condition. The results of Atrovastatin and Fenifibrate are shown in the table no: 1.

Table no: 1 Results of forced degradation studies of Atorvastatin and Fenofibrate

Degradation mechanism	% Assay		% Degradation		3^{rd} Point peak purity	
	Atorvastatin	**Fenofibrate**	**Atorvastatin**	**Fenofibrate**	**Atorvastatin**	**Fenofibrate**
Undegraded sample	101.0	101.0	-	-	0.99963	0.99979
Acid/1N HCL, 5ml, heated at 80^0 C for 2hrs	83.8	99.5	17.0	2.4	0.99911	0.99951
Base/0.5N NaOH, 5ml heated at 80^0 C for 2hrs	98.9	101.5	2.1	0.4	0.9974	0.99868
Peroxide/1.5% H_2O_2 heated at 80^0 C for 2hrs	92.4	98.3	8.5	2.9	0.99176	0.99957
Solid state						
Undegraded sample	101.0	101.9	-	-	0.99963	0.99979
Termal at 65^0 C for 48hrs	100.9	100.5	0.1	1.4	0.99904	0.99887
Photo stability degradation 1.2million lux hours 200watt hr/m^2	100.8	101.0	0.2	0.9	0.99989	0.99969
Humidity at 90% RH 168hrs	100.5	99.3	0.5	2.6	0.99988	0.99923

Linearity

A series of solutions were prepared using Atorvastatin and Fenofibrate working standards at the concentration levels for 50% to 150% of target concentration. The correlation coefficients of both the drugs were found to be greater than 0.999. The linearity parameters are shown in table 2 where as their calibration curves are shown in figure no:4 and 5 respectively.

Table no: 2 Linearity results

Parameter	Atorvastatin	Fenofibrate
Conc. Range (µg/ml)	5.003 - 60.03	144.225 - 480.750
Slope (m)	46460.47	40961.66
Intercept	14157.30	68004
Correlation coefficient	0.9988	0.99879
R^2	0.9976	0.9998

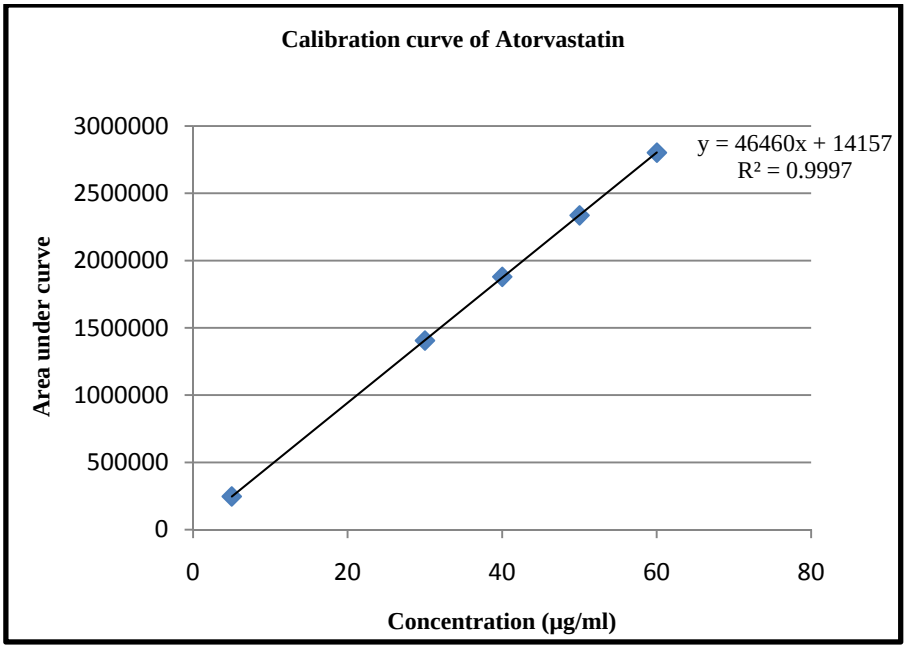

Figure 4: Calibration curve of Atorvastatin

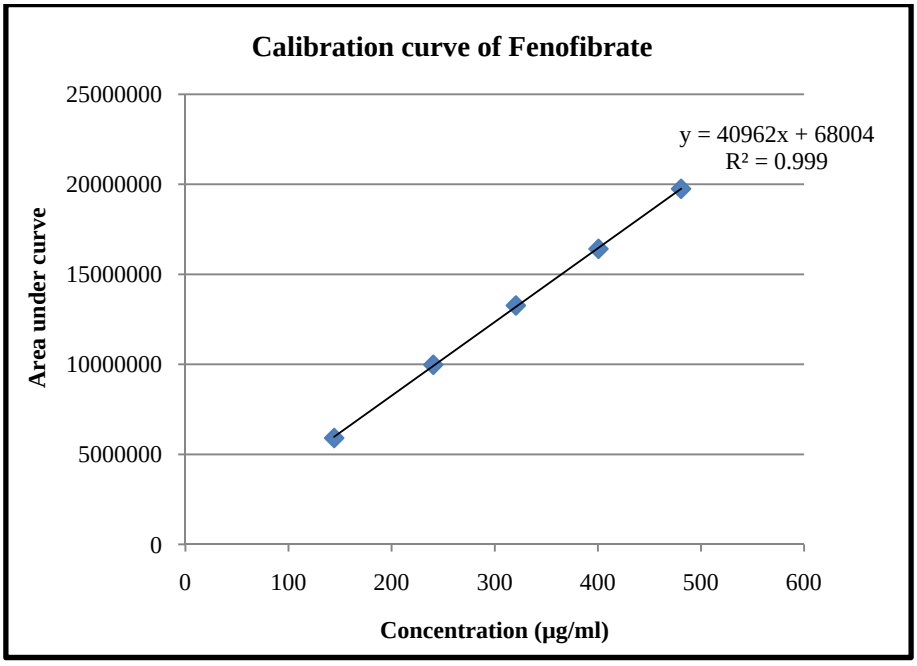

Figure 5: Calibration curve of Fenofibrate

Accuracy

A study of accuracy was conducted. Drug assay was performed in triplicate as per testmethod with equivalent amount of Atorvastatin andFenofibrate into each volumetric flask for each spike level to get the concentrations to 50%, 100% and 150% of the labeled amount and the average % recovery was calculated by injecting blank, placebo and the drug samples into the chromatogram. The results are shown in table no: 3.

Table no: 3 Recovery results of Atorvastatin and Fenofibrate

% Spike level	Amount added (mg/ml)*		Amound found (mg/ml)**		% Recovery***	
	Atorvastatin	**Fenofibrate**	**Atorvastatin**	**Fenofibrate**	**Atorvastatin**	**Fenofibrate**
50%	2.6	72.46	2.62	72.74	100.7	100.3
100%	10.576	145.8	10.55	145.61	99.7	99.8
150%	15.263	217.51	15.16	216.6	99.3	99.5
Overall Statistical Analysis						
		Mean	**SD**		**% RSD**	
Atorvastatin		99.9	0.721		0.720	
Fenofibrate		99.86	0.404		0.404	

* Average of Amount added **Average of Amount found ***Mean of % Recovery

Precision

Standard solutions was prepared as per the test method and injected six times for system precision where as six sample preparations individually using batch of FIBATOR tablets for intermediate precision were prepared and injected each solution into the chromatographic system. The values are depicted in the table no: 4.

Table no: 4 System and intermediate precision data

S.No	% Assay			
	System precision		**Intermediate precision**	
	Atorvastatin	**Fenofibrate**	**Atorvastatin**	**Fenofibrate**
1.	99.5	100.8	99.1	100.0
2.	98.6	100.0	98.1	100.1
3.	99.0	100.5	99.2	100.8
4.	99.9	100.9	101.9	104.0
5.	99.8	100.3	99.6	101.3
6.	99.3	100.6	98.8	100.6
Mean	99.35	100.516	99.45	101.13
SD	0.492	0.331	1.30	1.482
% RSD	0.49	0.328	1.3	1.46

Stability of the solution

The prepared standard and sample solutions were stored at refrigerator condition and were evaluated at initial, 12hrs, 24hrs and 48hrs respectively. The standard and sample solutions were found to be stable upto 48hrs under refrigerator conditions. The results are shown in table no: 5.

Table no: 5 Stability results

Standard solution: Overall % RSD				
Time Interval	**Overall % RSD of Atorvastatin**		**Overall % RSD of Fenofibrate**	
Initial	0.43		0.09	
12 hrs	0.62		0.19	
24 hrs	0.50		0.16	
48 hrs	0.65		0.15	
Sample solution: % Assay Difference				
Time Interval	**% Assay**		**Difference of % Assay**	
	Atorvastatin	**Fenofibrate**	**Atorvastatin**	**Fenofibrate**
Initial	99.2	99.7	-	-
12 hrs	99.2	99.1	0.0	0.6
24 hrs	98.7	98.5	0.5	1.2
48 hrs	99.4	98.5	0.2	1.2

Robustness

The system suitability study was analyzed for robustness by altering the flow rate, composition of mobile phase and temperature. There was no significant change observed indicating the method to be robust. The results of robustness are shown in table no: 6.

Table no: 6 Robustness results of Atorvastatin and Fenofibrate

Parameter		RSD for Peak Area		Theoretical Plates		Tailing Factor	
				Min. Observed value		**Max. Observed Value**	
		Atorvastatin	Fenofibrate	Atorvastatin	Fenofibrate	Atorvastatin	Fenofibrate
Flow variation (1.0ml/min)	**1.1ml/min**	0.10	0.18	5189.73	9342.93	1.29	1.21
	0.9ml/min	0.05	0.06	5921.21	10490.56	1.28	1.19
Temperature variation (30^0 C)	**25^0 C**	0.04	0.05	5623.20	10523.97	1.27	1.18
	35^0 C	0.08	0.03	5707.33	10313.25	1.29	1.19
Mobile phase composition variation (150:850)	**130:870**	0.48	0.47	5722.60	9990.70	1.26	1.19
	170:830	0.17	0.12	5780.71	10704.10	1.28	1.18
pH variation (2.5±0.2)	**2.3**	0.18	0.11	5617.70	10028.88	1.30	1.20
	2.8	0.13	0.10	5619.00	10028.88	1.29	1.20

Limit of detection and Limit of quantitation (LOD and LOQ)

Limit of detection and quantitation were evaluated based on statistical calculation to linearity results. LOD and LOQ of Atorvastatin were found to be 1.005µg/ml and 3.04µg/ml whereas, for Fenofibrate, it was 5.47µg/ml and 16.60µg/ml respectively.

CONCLUSION

The developed and validated analytical method was found to be specific and accurate as all the forced degradation parameters have passed 3rd point peak purity. Linearity of Atorvastatin and Fenofibrate were greater than 0.999. The method is robust as the method was unaffected by changing the chromatographic conditions. Therefore, the method developed can be adopted for the routine analysis of Atorvastatin and Fenofibrate in pharmaceutical formulations.

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