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METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF ATORVASTATIN CALCIUM AND FENOFIBRATE IN COMBINED TABLET DOSAGE FORM BY RP-HPLC

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Abstract

A simple, precise, accurate, rapid, RP-HPLC procedure is developed for the simultaneous determination of atorvastatin calcium and fenofibrate in tablet formulations. Chromatographic separation was achieved by isocratic mode technquies. The mobile phase used was a mixture of ammonium acetate (pH-4.5): acetonitrile: Tetra hydro furan (THF) in the ratio of (40: 50: 10 v/v/v) and Hypersil BDS C_{18} column as stationary phase was used. The detection of the tablet dosage form was carried out at 252 nm and flow rate employed was of 1 mL/min. The retention times for atorvastatin calcium and fenofibrate was found to be 2.713 min and 4.770 min respectively. The linearity was obtained in the concentration range of 6 to 14 µg/mL for atorvastatin calcium and 87-203 µg/mL for fenofibrate with a correlation coefficient value is found to be 0.998 for atorvastatin calcium and 0.999 for fenofibrate respectively. The LOD and LOQ for atorvastatin calcium was found to be 0.02 -0.07 µg/mL and fenofibrate was found to be 21.36- 64.72 µg/mL respectively. The results of the analysis were validated statistically and recovery studies confirmed the linearity, range, accuracy, precision, specificity and robustness. The developed method has been successfully used for the simultaneous estimation of both drugs in commercial formulations.

Keywords: Atorvastatin calcium, Fenofibrate, UV spectrometer, High performance liquid chromatography (HPLC).

Introduction

Atorvastatin calcium (ATR) (fig.1a) is chemically (3R, 5R)-7-[2-(4-Fluorophenyl)-5-(1-methylethyl)-3-phenyl 4-(phenyl amino carbonyl)-1*H*-pyrrol-1-yl]-3, 5-dihydroxy-heptanoic acid calcium salt[1]. It is a orally administered HMG–CoA

reductase inhibitor class of anti hyperlipidemic drug used to treat dyslipidemia and the prevention of cardiovascular disease.

Atorvastatin calcium (ATR) is a competitive inhibitor of HMG-CoA reductase. HMG-CoA reductase catalyzes the reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate, which is the rate-limiting step in hepatic cholesterol biosynthesis. Inhibition of the enzyme decreases cholesterol synthesis, increasing expression of low-density lipoprotein receptors (LDL receptors) on hepatocytes. This increases LDL uptake by the hepatocytes, decreasing the amount of LDL-cholesterol in the blood. Like other statins, ATR also reduces blood levels of triglycerides and slightly increases levels of HDL-cholesterol[2].

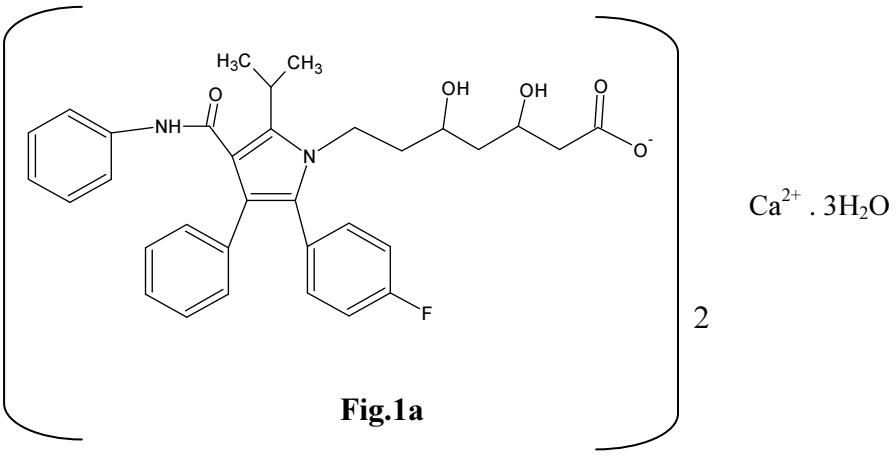

Fig.1a

Fenofibrate (FEN) (fig. 1b) is chemically propan-2-yl 2{4-[(4-chlorophenyl)-carbonyl] phenoxy}-2-methylpropanoate[3]. 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[4].

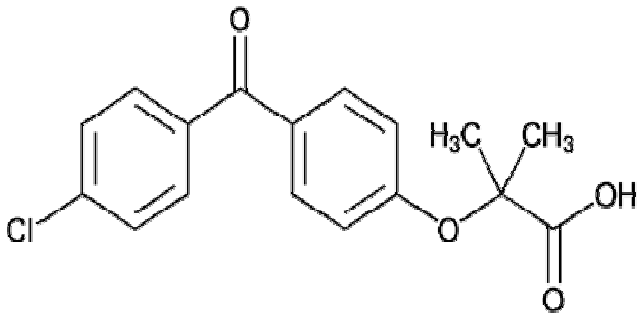

Fig.1b

Atorvastatin calcium and Fenofibrate combination therapy has improved patients Atherosclerotic vascular diseases (AVD) risk status significantly more in comparison to each drug alone. ATR is more effective in reduction of total

cholesterol level whereas FEN is more efficient in reduction of triglycerides. The combination had beneficial effects on oxidative stress and on vascular reactivity in hyperlipidemia[2, 4].

Different methods, few HPLC[5-8] and HPTLC[9] methods and few stability indicating[10-12] methods and spectroscopic methods[13, 14] are available for estimation of Atorvastatin calcium and Fenofibrate combination. Literature survey reveals that more retention times and so long run time was required for both the drugs and use of cumbersome methods were reported. Therefore the present study was aimed to reduce the run time therefore reduce the run time and time of analysis. The present work describes development of simple, precise and accurate isocratic reverse phase HPLC method for simultaneous estimation of ATR and FEN in tablet formulation. The developed method has been validated according to ICH guidelines[15, 16].

Materials and Methods

Instrumentation

An isocratic HPLC Cyber lab (Salo Terrace, Millbury, USA) with UV detector equipped with Hypersil BDS C_{18} column (250 mm x 4.6 mm i.d, 5µ particle size) was employed for the study.

Reagent and Solution

Acetonitrile HPLC grade, Methanol HPLC grade, water HPLC grade were purchased from Merck chemicals, India. Ortho-phosphoric acid and Tri ethyl amine for pH adjustment.

Chromatographic conditions

For chromatographic analysis, Hypersil BDS C_{18} column (250 mm x 4.6 mm i.d, 5µ particle size) was used. Separation was carried out by isocratic elution. The solvent system was a mixture of Ammonium acetate buffer (pH 4.5 with ortho phosphoric acid): Acetonitrile: Tetra hydro furan. (40:50:10 % v/v/v). It was filtered under vacuum from 0.45 membrane filter and degassed in ultrasonic bath for 15 min before passing through the instrument. The injection volume was 20 µl and the flow rate was 1ml/min. UV detection was carried out at 252 nm.

Preparation of Mobile Phase

1000ml of mobile phase was prepared by mixing 400 ml Ammonium acetate buffer (pH 4.5), 500 ml of Acetonitrile and 100 ml of Tetra hydro furan. pH adjusted to 4.5 with ortho phosphoric acid.

Filtration of Mobile Phase

The degassed mobile phase was filtered through 0.45µn filter to avoid the column clogging due to smaller particles.

Selection of mobile phase

Different mixtures of mobile phase with different ratios were selected and their chromatograms were recorded. From this Ammonium acetate buffer (pH 4.5 with ortho phosphoric acid): Acetonitrile: Tetra hydro furan. (40:50:10 % v/v/v) was selected as mobile phase, since these two drugs were eluted with sharp peak and with better resolution. Hence this mobile phase was used to optimize the chromatographic conditions. The detection wavelength was measured by scanning the 10 mg/ml solution of Atorvastatin calcium and Fenofibrate in mobile phase, in UV spectrophotometry, overlaid spectra and the wavelength of maximum absorption was selected as 252 nm.

Preparation of standard stock solution

ATR standard stock solution

Weighed accurately 5 mg of ATR and taken in a 50 mL standard flask. To it 25 mL of the mobile phase was added and sonicated for 5 min. to dissolve the drug. The volume was made up to 50 mL with the mobile phase. It consist of 100 µg/mL of ATR and filtered through 0.45 µm membrane filter.

FEN standard stock solution

Weighed accurately 72.5 mg of FEN and taken in a 50 mL standard flask. To it 25 mL of the mobile phase was added, sonicated for 5 min. to dissolve the drug. The volume was made up to 50 mL with mobile phase. It consists of 1450 µg/mL of FEN, filtered through 0.45 µm membrane filter.

Quantification of Atorvastatin calcium and Fenofibrate

Twenty tablets were finely powdered and an accurately weighed sample of powdered tablets equivalent to ATR (10 mg) and FEN (145 mg) were transferred to a 50 mL volumetric flask and dissolved in Mobile Phase. The solution was shaken well and allowed to stand for 15 min with intermittent sonication to ensure complete solubility of drug. The contents were made up to the mark with Mobile Phase and filtered through a 0.45µ membrane filter. From the filtrate, dilution was made in a 10 mL volumetric flask to get 10µg/ mL of Atorvastatin calcium and 145µg/mL of Fenofibrate respectively

with diluents. The peak area measurements were done by injecting each sample three times and the amount of Atorvastatin calcium and Fenofibrate were calculated.

Method Validation

As per the International Conference on Harmonization (ICH) guidelines. The method validation parameters such as specificity, linearity, precision, accuracy, limit of detection/quantization and robustness were optimized.

Linearity

To establish linearity, the stock solutions were prepared (1000 µg/mL of both ATR and FEN) using Mobile Phase as the solvent, again from the stock solution further dilutions were made to yield solutions in the concentration range of 6-14 µg/mL of Atorvastatin calcium and 87-203µg/mL of Fenofibrate. 20 µl of each solution was injected and records the chromatogram at 252 nm.

The chromatogram optimized given in (Figure-2) and their system suitability parameters were given in (Table -1), the calibration curve was plotted using concentration against peak area. The procedure was repeated for three times. The correlation coefficient was found to be above 0.998 and 0.999 Atorvastatin calcium and Fenofibrate. The optical characteristics of Atorvastatin calcium and Fenofibrate shown in (Table-2).

Figure-2: Optimized Chromatogram for Atorvastatin calcium and Fenofibrate

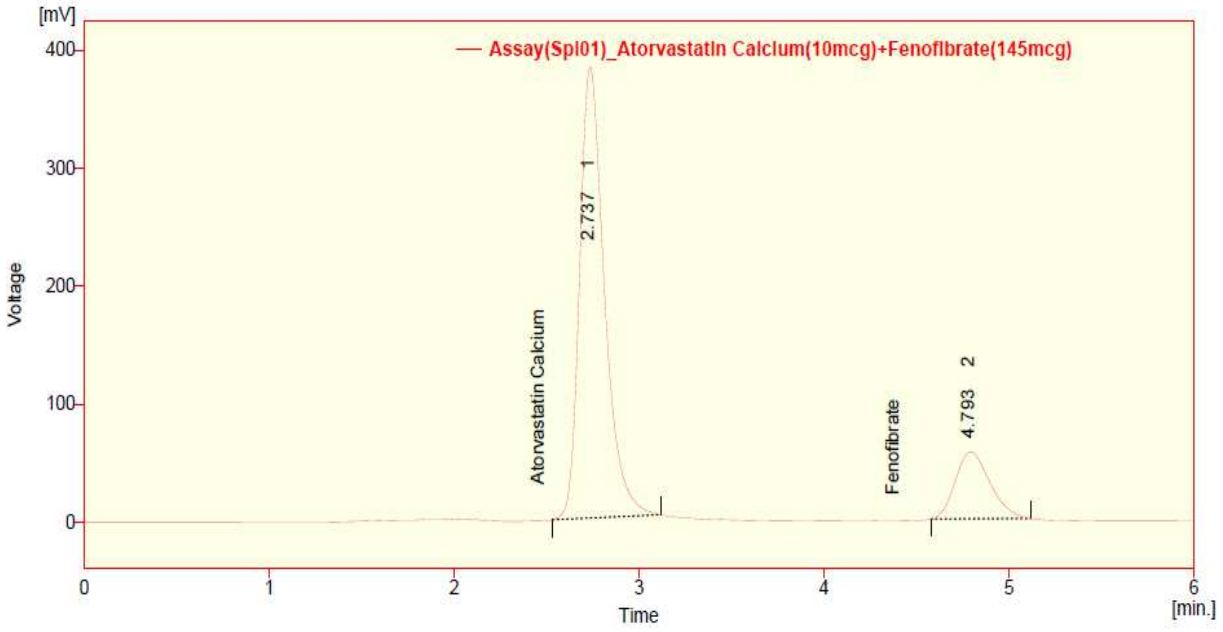

Table-1: System Suitability Parameters for the Optimized Chromatogram by RP-HPLC.

Parameters	Atorvastatin calcium	Fenofibrate
Retention time	2.737	4.793
Peak area	3556.484	740.028
Tailing factor	1.625	1.396
Theoretical plates	2020	2980
Resolution	Between ATR and FEN	6.915

Table-2: Optical Characteristics.

Parameters	Atorvastatin calcium	Fenofibrate
Detection Wavelength(nm)	252	252
Beers law limit (µg mL $^{-1}$)	6-14	87-203
Regression equation (y=mx+c)	y = 435.1x - 559.1	y = 7.085x - 187.4
Correlation coefficient (r^2)	0.998	0.999
Slope (m)	435.1	7.085
Intercept (c)	559.1	187.4
LOD (µg mL^{-1})	0.02	21.36
LOQ (µg mL^{-1})	0.07	64.72

Precision

Precision was determined by analysing standard preparations of Atorvastatin calcium (10 µg/mL), Fenofibrate (145µg/mL), for six times. The chromatograms were recorded and the results were summarized in (Table 3).

Table -3: Quantification of tablet formulation (ATOCOR F TAB) BY RP-HPLC.

Injection	Atorvastatin calcium		Fenofibrate	
	RT	Area	RT	Area
1	2.61	3357.977	4.607	736.616
2	2.653	3435.285	4.637	756.095
3	2.633	3435.649	4.610	740.222
4	2.63	3487.630	4.603	753.7
5	2.62	3403.080	4.587	734.433
6	2.633	3408.658	4.610	727.074
Average	2.6298	3421.380	4.609	741.357
SD	0.0145	43.134	0.016	11.360
%RSD	0.55	1.26	0.35	1.53

Accuracy

The accuracy of the method was performed by recovery studies to the pre analyzed formulation, a known quantity of Atorvastatin calcium and Fenofibrate working standard solutions were added at different levels, 80% (10 µg/mL for ATR; 145 µg/mL for FEN), 100% (12µg/mL for ATR; 174 µg/mL for FEN), and 120% (14 µg/mL for ATR; 203 µg/mL for FEN), (Three replicates each) of the theoretical concentrations were injected the solutions and the chromatograms were recorded.

The percentage recovery was found to in the range between 100.79-101.47% for Atorvastatin calcium and 100.80-101.14% for Fenofibrate. Recovery data was given in (Table 4). The results of recovery revealed that no interference was produced due to the excipients used in formulation. Therefore developed method was found to be accurate.

Table-4: Recovery data of Atorvastatin calcium and Fenofibrate

Recovery Level (%)	Drug	Conc. of drug (µg/ml)		% Recovery	%Mean Recovery
		Drug Taken	Std. drug added		
80	Atorvastatin calcium	2	8	100.72	100.99
100		2	10	101.47	
120		2	12	100.79	
80	Fenofibrate	29	116	100.29	100.7
100		29	145	101.14	
120		29	174	100.80	

Robustness

The robustness of the method was determined as per USP guidelines under different conditions including change in flow rate and wavelength. The chromatograms were recorded and the results were summarized in (Table 5).

Table-5: Results of robustness by variations in flow rate and wavelength.

Parameter	Value	Atorvastatin calcium		Fenofibrate	
		RT	TF	RT	TF
Flow rate	0.8 mL/min	3.133	1.714	5.480	1.538
	1.0 mL/min	2.713	1.625	4.770	1.396
	1.2 mL/min	2.373	1.586	4.160	1.442

Wavelength	250 nm	2.683	1.625	4.713	1.511
	252 nm	2.713	1.625	4.770	1.396
	254 nm	2.707	1.677	4.740	1.438

Conclusion

The present method was a sensitive, precise, and accurate HPLC method for the analysis of Atorvastatin calcium (ATR) & Fenofibrate (FEN). To optimize the mobile phase, various combinations of buffer and organic solvents were used on Hypersil BDS C_{18} column. Then the mobile phase containing a mixture of Ammonium acetate buffer (pH 4.5): ACN: THF (Tetra hydro furan) in the ratio of 40:50:10, (v/v/v) was selected at a flow rate of 1.0 mL/min for developing the method and the peaks with good shape and resolution were found resulting in short retention time, baseline stability and minimum noise. The retention times of ATR and FEN were found to be 2.713 min and 4.770 min respectively. Quantitative linearity was obeyed in the concentration range of 6-14 and 87-203 µg/mL of ATR and FEN respectively. The limit of detection and limit of quantitation were found to be 0.02 µg/mL and 0.07µg/mL (ATR); 21.36 µg/ mL and 64.72 µg/mL (FEN) respectively, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulations didn't interfere with the estimation of the drugs by the proposed HPLC method.

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References

1. Indian Pharmacopoeia, Published by The Indian Pharmacopoeia commission, Ghaziabad, 2010. Vol. II, p.849.
2. R. Vijayamirtharaj, J. Ramesh, B. Jayalakshmi, Hanas Bin Hashim. Development and validation of RP-HPLC method for the simultaneous estimation of telmisartan and atorvastatin calcium in tablet dosage forms. International Journal of Comprehensive Pharmacy, 2010, 4(3), pp1-4.

3. Indian Pharmacopoeia, Published by The Indian Pharmacopoeia commission, Ghaziabad, 2010, Vol. II, p.1337.
4. R.K. Jat, S. Sharma, R.C. Chippa, Singh Rambir, Alam Imran. Quantitative estimation of fenofibrate in bulk drug and tablets by U.V visible spectroscopy. Journal of Drug Delivery & Therapeutics, 2012, 2(3), pp129-131.
5. T. Deepan, K. Paul ambethkar, G. Vijayalakshmi, M. D. Dhanaraju. Analytical method development and validation of RP-HRLC for estimation of atorvastatin and fenofibrate in bulk drug and tablet dosage forms. European Journal of Applied Sciences, 2011, 3(2), pp35-39.
6. N. Jain, R. Raghuwanshi, D. Jain. Development and validation of RP-HPLC method for simultaneous estimation of atorvastatin calcium and fenofibrate in tablet dosage forms. Indian Journal of Pharmaceutical Sciences, 2008, 70(2), pp263-265.
7. S. D. Bhinge, S. M. Malipatil, A. Jondhale, R. Hirave, A. S. Savali. A New approach to the RP-HPLC method for simultaneous estimation of atorvastatin calcium and fenofibrate in pharmaceutical dosage forms. E-Journal of Chemistry, 2012, 9(3), pp1223-1229.
8. M. Jagadeeswaran, N. Gopal, B. Jayakar, T. Sivakumar. A Validated RP-HPLC method for simultaneous determination of atorvastatin and fenofibrate in bulk and its dosage form. International Journal of Pure and Applied Chemistry, 2012, 7(4).
9. P. B. Deshpande, G. Shridharan, L. D. Anandi, M. C. Jadhav, S.V.G. Damle. Validated method development for estimation of atorvastatin calcium and fenofibrate in fixed dose combination by HPTLC. 2008, 48(1), pp120-126.
10. M. Sudheer, M. Siva Prakash, G. Rajashekar, K. Vani, V. Hima, P. Ramalingam, Y. Reddy, Padmanabha. A Stability indicating HPLC method with diode array detection for the determination of atorvastatin calcium and fenofibrate in commercial tablets. Journal of Pharmacy Research, 2011, 4(9), pp3033.
11. V. D. Prakash, N. Anusha, H. Sanayaima, N. Surendra, V.V.L.N. Prasad. Stability indicating high performance liquid chromatographic assay for the simultaneous determination of atorvastatin calcium and fenofibrate in tablet dosage forms. International Journal of Pharmacy and Industrial Research, 2012, 2, pp331-339.
12. A.A. Kadav, D.N. Vora. Stability indicating UPLC method for simultaneous determination of atorvastatin, fenofibrate and their degradation products in tablets. Journal of Pharmaceutical and Biomedical Analysis, 2008, 48(1), pp120–126.

13. E. Bose, Subhash Chandra, C. Krishna, Murali, S. Basha, Mahaboob, N. Ramesh, D. H. Theja. Simultaneous spectrophotometric estimation of atorvastatin and fenofibrate in bulk and pharmaceutical dosage forms by absorbance ratio method (Q-analysis). Journal of Pharmacy Research, 2011, 4(9), pp2933.
14. P. N. Dhabale, D. S. Gharge. Simultaneous spectrophotometric estimation of atorvastatin and fenofibrate in bulk drug and dosage form by using simultaneous equation method. International Journal of Chemtech Research, 2010, 2(1), pp325-328.
15. Validation of Analytical Procedures Q2B. International Conference on Harmonization, Federal Register, 1997, 62, 27463–27467.
16. Validation of Analytical Procedures Q2 (R1). International Conference on Harmonization, 2005, 1-6.

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