DEVELOPMENT AND VALIDATION RP- HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ATORVASTATIN AND FENOFIBRATE IN BULK AND PHARMACEUTICAL FORMULATIONS

ABSTRACT:

A reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of atorvastatin and Fenofibrate in bulk and pharmaceutical dosage forms. The determination was performed by using Waters Symmetry C18 (250×4.6mm×5µ) as column stationary phase and Methanol: Acetonitrile: Water in the ratio of (70:10:20 %v/v) as mobile phase. The flow rate of mobile phase was optimized as 1mL/min and effluents were monitored at 274nm. The retention time of atorvastatin and Fenofibrate were found as 4.15 min and 8.10 min respectively. The method shows linearity concentration rangesbetween 4-30 µg/mL and 80-400 µg/mL respectively. The developed method was validated for specificity, precision, linearity, accuracy, robustness, Ruggedness, LOD and LOQ. Recovery of atorvastatin and Fenofibrate in formulations was found to be 100.52% and 99.92% respectively which conforms the non-interferences of the excipients in the formulation. the proposed RP- HPLC method can be used for the simultaneous determination of these two drugs in bulk and formulation.

KEYWORDS: Atorvastatin calcium, Validation, Fenofibrate, robustness, validation

INTRODUCTION

Atorvastatin calcium 1-4 (AT) is (β R, δ R)-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methyl ethyl)-3-phenyl-4-((phenyl amino)carbonyl)-1H-pyrrole-1-hepatonoic acid, a HMG CoA reductase inhibitor. Fenofibrate (FB) is 2-[4-(4-chlorobenzoyl) phenoxy]-2-methylpropanoic acid, 1-methylethyl ester, it is a lipid lowering agent.(Bhinge, Malipatil, 2012) Literature survey reveals that, few HPLC and HPTLC methods have been reported for simultaneous estimation of ATR and FB as well as in combination with other drugs. (N. Jain 2008) has reported HPLC method for simultaneous estimation of ATR and FB using Methanol-Acetonitrile: Phosphate Buffer pH 5.0 (45:25:30 V/V) as eluting solvent. The

methods reported by (Kadav2008) were excluding the internal standard, which was found to be the limitation of the method. Literature survey also revealed number of UV-VIS spectroscopic(Krishna, Mudraboina 2021),NP-HPLC,RP-HPLC(M.T. Zaman, S.A. Khan 2009),GC and some electro-analytical methods for estimation of these drug alone or in combination with other drugs(6-17).

MATERIALS AND METHODS

Quantitative HPLC was performed on a high-performance liquid chromatograph -Waters HPLC system connected with PDA Detector and Empower2 Software. The drug analysis data were acquired and processed using Empower2 software running under Windows XP on a Pentium PC and C18column of dimension 250 × 4.6, 5µm particle size.

Preparation and Selection of mobile phase:

The preliminary isocratic studies on a reverse phase C18 column with different mobile phases like Acetonitrile, Methanol and Distilled water different buffers were tried. After some trials the mobile phase was optimized as follows.

A mixture of 70 volumes of Methanol, 10 volumes of Acetonitrile and 20 volumes of Water was used as mobile phase. The mobile phase was filtered through 0.45µ membrane filter to remove all fine particles and ultra-sonicated for 10min to remove dissolved gases.(Choudhari, Vishnu &Nikalje, Anna. 2010)

Preparation of standard solution:

Preparation of standard stock solutions of Fenofibrateand atorvastatin:

Weigh accurately 10 mg of Fenofibrate and atorvastatin (Working standard) in to a 100 ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol this solution was further diluted to get desired concentration range.

Preparation of Sample Solution:

Weigh accurately powder equivalent to 16 µg/ml of Fenofibrate and 1mg of atorvastatin and transfer to 10ml volumetric flask. Then add enough mobile phase to dissolve the content by vigorous shaking or subjected to sonication and make up the final volume up to the mark with mobile phase. Then filter the resulting solution through whattman filter paper.

From the above solution pipette out required aliquots and dilute with mobile phase to prepare the solution containing the concentration of 160 µg/ml of Fenofibrate and10µg/ml of atorvastatin. This solution was used for recording the chromatogram.

Chromatographic Conditions:

The mobile phase methanol: Acetonitrile : Water in the ratio of 70: 10: 20 v/v was pumped at a flow rate of 1 mL /min through the waters symmetry (250×4.6×5µ) column at room temperature.The mobile phase was degassed prior to use under vacuum by filtration through a 0.45µ membrane filter. Both drugs showed high absorbance values at 274 nm, which was selected as wavelength for further analysis.

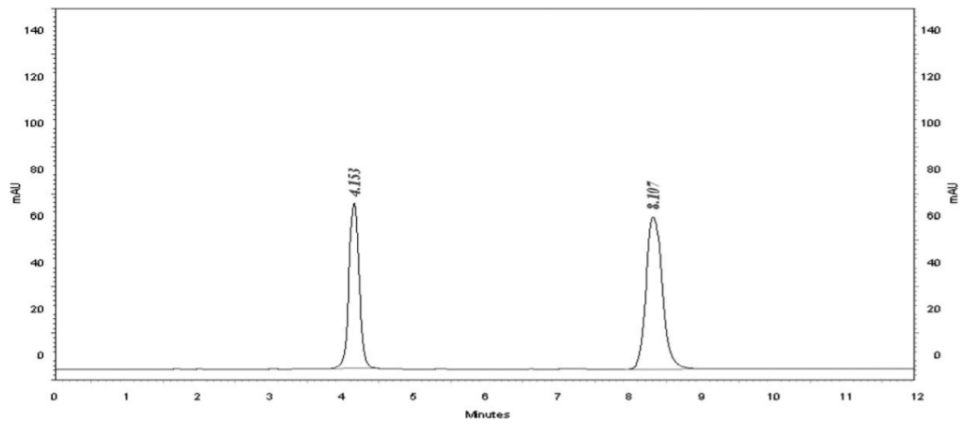

Figure 1: Chromatogram ofFenofibrate and Atorvastatin

System Suitability Study:

System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentrationof atorvastatin and Fenofibrate. Various chromatographic parameters such as retention time, peak area tailing factor, theoretical plates (Tangent) of the column and resolution between the peaks were determined and the method was evaluated by analysing these parameters. (Table 2)

Validation of Developed Analytical Method.

Linearity:

Linearity of the method was determined by constructing calibration curves. Standard solutions of atorvastatin and Fenofibrate of different concentrations level were used for this purpose. Each measurement was carried out in six replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients

Accuracy (Recovery Studies):

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. Known amounts of standard drugs were added to pre-analyzed samples and were subjected to the proposed HPLC method. The measured value was obtained by recovery test. Spiked amount of both the drugs were compared against the recovery amount. % recovery was 100.52% for atorvastatin and 99.92% for Fenofibrate. (Table 3 and 4)

Precision:

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation

Robustness of Method:

To evaluate the robustness of the developed RP-HPLC method the prepared solution as per the test method was injected at different variable conditions like using different conditions flow rate and wavelength. (Table5)

Limit of detection and limit of quantification:

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were calculated using the following equations as per International Conference of Harmonization (ICH) guidelines. (Table 6)

$LOD = 3.3 \times \alpha / S$

$LOQ = 10 \times \alpha / S$

RESULTS AND DISCUSSION:

Development and optimization of the methodColumn chemistry, solvent type, solvent strength, detectionwavelength and flow rate were varied to determine thechromatographic conditions giving the best separation. Separationwith good resolutions was studied on different type's columns (C8 andC18). The mobile phase conditions were optimized so that thecomponents were not interfered from the solvent and excipients.Several buffer systems at different pH values were trailed in variousratios with methanol, and acetonitrile as mobile phase. Mobile phase and flowrate selection was based on peak parameters like area, tailing,theoretical plates, capacity factor and resolution.Decisively after several experimental trials, the best result was obtainedby use of phase methanol: Acetonitrile: Water in the ratio of 70: 10: 20 v/v with 1.0 mL/minflow rate.

System suitability parameters like number of plates, asymmetry factor, resolution was found within the specified values suggested by regulatory guidelinesseen in table 1.

Drug		**Retention time (min)**	**Peak area**	**Theoretical plates**	**Tailing factor**	**Resolution**
Atorvastatin	**Mean**	4.148	5205.26	4046.5	1.58	
	SEM	0.0110	2.9699	0.9955	0.0360	
	%RSD	0.17	0.88	1.72	0.020	3.7
Fenofibrate	**Mean**	8.104	6337.24	6532.13	1.78	
	SEM	0.0017	0.2483	0.4000	0.0152	
	% RSD	0.0370	0.0067	0.0106	1.5118	

Table 1:Details of system suitability studies

The developed method was validated as per the parameters like System suitability parameters, linearity, precession, accuracy, robustness, and the values all above parameters are within the limit so the developed method was validated according to ICH guidelines. Linearity was determined based on value of the correlation coefficient for standard preparations of atorvastatin and Fenofibrate which was found to be 0.999 and 0.999 respectively. The relationship between the concentration of atorvastatin and Fenofibrate and area atorvastatin and Fenofibrate was linear in the range examined since all points lie in a straight line and the correlation coefficient was well within limits. Limit of Detection and

limit of quantitation was calculated based on the values of slope obtained from calibration curve. (Table2)

Table 2: Results of LOD and LOQ

Parameter	Atorvastatin	Fenofibrate
LOD	0.32	58
LOQ	0.76	73

Precision of the method for intra- day study was evaluated based on relative standard deviation and Percentage relative standard deviation (%RSD) which was found to be less than 2% for within a day variation, which proves that method is precise. (Table 3)

Table 3: Results of Precision study

Atorvastatin	Fenofibrate	Atorvastatin	Fenofibrate
5	80	5.07	80.08
5	80	5.08	80.12
5	80	5.04	80.07
5	80	5.06	80.14
5	80	5.03	80.19
5	80	5.02	80.05
	Mean	5.05	80.10
	SEM	0.0096	0.0212
	RSD	0.0236	0.0519
	%RSD	0.4686	0.0648

Accuracy of the developed method was evaluated based on recovery studies and mean percentage recovery value for each drug was found within 98-102% range which shows that there is no interference of excipients on the results. The mean percentage recovery of Atorvastatin and Fenofibrate was 100.53% and 99.92% respectively.(Table4 and 5)

Table 4: Results of Accuracy for Atorvastatin:

Amount of sample taken (µg/ml)	Amount of standard added (µg/ml)	% of Std. added	Amount recovered (µg/ml)	% Amount recovered*	% RSD
10	08	80	7.94	99.25	0.74
10	10	100	10.17	101.7	1.25
10	12	120	12.08	100.66	0.97

Table 5: Results of Accuracy for Fenofibrate:

Amount of sample taken (µg/ml)	Amount of standard added (µg/ml)	% of Std. added	Amount recovered (µg/ml)	% Amount recovered*	% RSD
16	12.8	80	12.81	100.07	1.22
16	16	100	15.97	99.81	1.08
16	19.2	120	19.18	99.89	0.59

Robustness of the method was evaluatedby some small and deliberate changes in the operating conditions and effect was evaluated with relative standard deviation. Values of relative standard deviation shown in table 5 suggests that there is no major effect of change in operating conditions

Table 6: Results of Robustness

Robustness		Atorvastatin	Fenofibrate
% RSD (Rt)		0.37	0.28
Area*	Change in Flow rate	4205.27	6393.67
	Change Wavelength	4200.89	6389.54

Developed method was used for analysis of commercial formulation and amount of drug obtained by the method was checked with the label claim amount and assay value was found to be 99.50 and 99.87% for atorvastatin and fenofibrate respectively. (Table 6)

Table 7Analysis of commercial formulation

Drug	Label Claim (mg/tab)	Amount Recovered*	Assay (%w/w)	%RSD
Atorvastatin	10	9.95	99.50	0.254
Fenofibrate	160	159.65	99.78	0.574

Conclusion

The developed method gave good resolution between atorvastatin and fenofibrate with short analysis time and high efficiency and complies with all system suitability test specifications of USP. The use of C18 column in the present work has indicatedbetter elution of analytes with good resolution, improved plate count and capacity factor.

References:

1.S. D. Bhinge, S. M. Malipatil,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), 1223-1229

2. Nagaraj, Vipul K, Rajshree M. Simultaneous quantitative resolution of atorvastatin calcium and fenofibrate in pharmaceutical preparation by using derivative ratio spectrophotometry and chemometric calibrations. Anal Sci. 2007 Apr;23(4):445-51. doi: 10.2116/analsci.23.445. PMID: 17420550.

3. Jain N, Raghuwanshi R, Jain D. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Atorvastatin Calcium and Fenofibrate in Tablet Dosage Forms. Indian J Pharm Sci. 2008 Mar-Apr;70(2):263-5. doi: 10.4103/0250-474X.41473.

4. 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, Volume 48, Issue 1, 2008, Pages 120-126, ISSN 0731-7085, https://doi.org/10.1016/j.jpba.2008.05.018

5. Nakarani NV, Bhatt KK, Patel RD, Bhatt HS. Estimation of atorvastatin calcium and fenofibrate in tablets by derivative spectrophotometry and liquid chromatography. J AOAC Int. 2007 May-Jun;90(3):700-5.

6 Said A Hassan, Hany W.Darwish, Maissa Y Salem, Development and Validation of HPoint Standard addition method applied for the analysis of binary mixture of Amlodipine and Atorvastatin, International Journal of Pharma and bio Sci 4(2),230-243, Apr (2013)

7. Ertürk S, SevinçAktaş E, Ersoy L, Fiçicioğlu S. An HPLC method for the determination of atorvastatin and its impurities in bulk drug and tablets. J Pharm Biomed Anal. 2003 Dec 4;33(5):1017-23. doi: 10.1016/s0731-7085(03)00408-4.

8. Zaheer, Zahid & Farooqui, Mazahar& Mangle, AA &Nikalje, Anna. (2008). Stability-indicating high performance liquid chromatographic determination of atorvastatin calcium in pharmaceutical dosage form. African Journal of Pharmacy and Pharmacology. 2. 204-210.

9. M.T. Zaman, S.A. Khan, A. Arora, O. Ahmad, METHOD DEVELOPMENT AND VALIDATION OF FENOFIBRATE BY HPLC USING HUMAN PLASMA, Electron J Biomed 2009;3:41-54.

10. Shah DA, Bhatt KK, Mehta RS, Baldania SL, Gandhi TR. Stability Indicating RP-HPLC Estimation of Atorvastatin Calcium and Amlodipine Besylate in Pharmaceutical Formulations. Indian J Pharm Sci. 2008;70(6):754-760. doi:10.4103/0250-474X.49117

11. Shah D A, Bhatt K K, Mehata R S, Baldania S L and Gandhi T R, Ind. J Pharm Sci. 2008, 70(6), 754. 16. Shah D A, Bhatt K K, Mehata R S, Shankar M B, Baldania S L and Gandhi T R, Ind. J Pharm Sci. 2007, 69(4), 701.

12. P. Mishra, Alka Gupta and K. Shah, Simultaneous estimation of atorvastatin calcium and amlodipine besylate from tablets, Indian J Pharm Sci, 2007, 69 (6): 831-833

13. Panchal HJ, Suhagia BN, Simultaneous determination of Atorvastatin calcium and Ramipril in capsule dosage forms by high performance liquid chromatography and high-performance thin layer chromatography. J AOAC Int. 93(5):1450-7, Sep-Oct (2010)

14. Potdar, V.H., Mule, V.S., Pishawikar, S.A., Jadhav, S.D., Thamake, S.L., RP-HPLC method for simultaneous estimation of atorvastatin calcium and ramipril from plasma,Ars Pharmaceutica., 52(2): 14-19 (2011).

15. Panchal, H.J., Suhagia, B.N., Patel, N.J., Rathod, I.S., Patel, B.H., Simultaneous estimation of atorvastatin calcium, ramipril and aspirin in capsule dosage form by RPLC, Chromatographia, 69(1-2): 91- 95(2009).

16. Mohammadi A, Rezanour N, Ansari Dogaheh M, GhorbaniBidkorbeh F, Hashem M, Walker RB. A stability-indicating high performance liquid chromatographic (HPLC) assay

for the simultaneous determination of atorvastatin and amlodipine in commercial tablets. J Chromatogr B AnalytTechnol Biomed Life Sci. 2007 Feb 1;846(1-2):215-21. doi: 10.1016/j.jchromb.2006.09.007. Epub 2006 Sep 28..

17. Choudhari, Vishnu &Nikalje, Anna. (2010). Simultaneous Estimation of Atorvastatin, Ezetimibe and Fenofibrate in Pharmaceutical Formulation by RP-LC-PDA. Pharmaceutical Analytica Acta. 1. doi:10.4172/2153-2435.1000111. 10.4172/2153-2435.1000111.

18. Sultana, Najma &Arayne, Mohammed & Naveed, Dr.Safila. (2011). Validated Method for the Simultaneous Determination of Lisinopril, Pravastatin, Atorvastatin and Rosuvastatin in API, Formulations and Human Serum by RP-HPLC. Chinese Journal of Chemistry. Chinese Journal of Chemistry 29, 1216—1220. doi/10.1002/cjoc.201190226

19. Krishna, Mudraboina& Sandhya, B &Huidrom, Sanayaima& Haque, M Akiful& Bakshi, Vasudha &Mudraboina, Johnny &Krishna,. (2019). DEVELOPMENT AND VALIDATION OF STANDARD ADDITION UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF ATORVASTATIN AND FENOFIBRATE IN BULK AND PHARMACEUTICAL DOSAGE FORM. IJIPSR / 2 (11), 2014, 2741-2748

20. Godge Ganesh Raosaheb, Garje Mahesh Arjun, VALIDATED SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF FENOFIBRATE AND ATORVASTATIN IN SYNTHETIC MIXTURE AND IN BULK TABLET DOSAGE FORM, WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH, wjpmr, 2020,6(7), 170-176

21. Doménech-Carbó, Antonio, de Carvalho, Leandro M., Martini, Mariele and Cebrián-Torrejón, Gerardo. "Voltammetric/amperometric screening of compounds of pharmacological interest" Reviews in Analytical Chemistry, vol. 33, no. 3, 2014, pp. 173-199. https://doi.org/10.1515/revac-2013-0027

22. ICH, Topic Q2A, Validation of analytical procedures: methodology.PMP/ICH/281/95.