



METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF FOLIC ACID, ATORVASTATIN AND FENOFIBRATE IN PHARMACEUTICAL DOSAGE FORMS BY UPLC

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ABSTRACT

Key Words

Folic acid,
Atorvastatin,
Fenofibrate
UPLC,
Validation



The present study is carried out using the UPLC as the analytical technique in developing and validating an accurate, precise, linear and robust analytical method for the simultaneous estimation of Folic acid, Atorvastatin and Fenofibrate in tablets. The method is optimized with a mixture of Buffer and Acetonitrile in the ratio of 50:50 (V/V) as mobile phase and Agilent SB C8 (2.1 X100) mm, 1.8 μ m as stationary phase. The Chromatographic peaks were detected and measured at 210nm. The retention times of Folic acid, Atorvastatin and Fenofibrate were found to be 1.35, 1.56 and 2.63 respectively. The developed method was demonstrated to access its suitability for meeting its intended purpose by the Validation with a set of validation parameters as per ICH and USP guidelines. The method is found to be precise with %RSD - 0.5, 1.0 and 0.6 for Folic acid, Atorvastatin and Fenofibrate respectively: accurate with the recoveries of 98.67 to 101.20, 99.57 to 99.97 and 99.24 to 100.18% for Folic acid, Atorvastatin and Fenofibrate respectively. The method is proved to be linear from the conc.1.25 to 7.50ppm for Folic acid, 2.50 to15.00 ppm for Atorvastatin and 1.00 to 6.00ppm for Fenofibrate with the correlation coefficient of 0.9988, 0.9991 and 0.9989 respectively. Hence the developed method could be used for the routine analysis purpose in the evaluation of Folic acid, Atorvastatin and Fenofibrate tablets.

INTRODUCTION:

Folic acid is synthetic vitamin and Hydrophilic vitamin (B9) which is essential for nutrient supplement for body. Folic acid is used for preventing and treating low blood levels of folate. One of the important coenzyme of the haemopoietic systems that controls the generation of ferrohaeme in body. Chemically it is 4-(2amino - 4 -

hydroxypteridin-6-yl) methyl amino benzoyl-l-glutamic acid. Atorvastatin calcium is ([R-(R*, R*)]-2-(4-fluorophenyl)- β , δ -dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate) and molecular formula is (C₆₆H₆₈CaF₂N₄O₁₀). An antihyperlipidemic agent (Biopharmaceutical classification system)

belongs to the class statins. It is widely used in the treatment of hypercholesterolemia as it decreases the levels of low density lipoproteins (LDL-C) and triglycerides (TGs) levels to a major extent and slightly increases the high density lipoprotein levels (HDL-C). It is insoluble in aqueous solutions having a pH 4 and below. Structure of Atorvastatin given in Fig No.2.

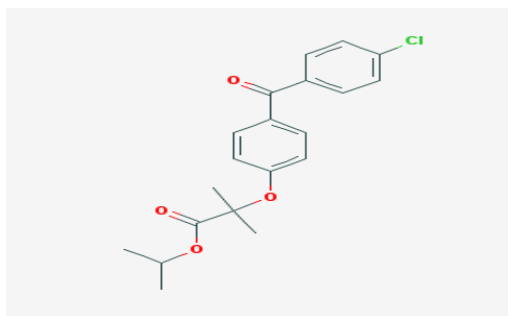


Fig.1: Structure of Folic acid

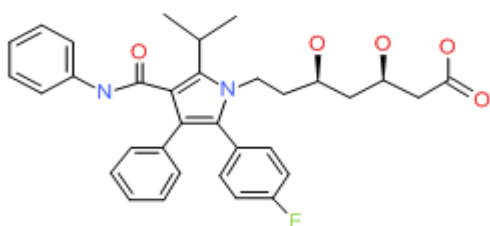


Fig 2: Structure of Atorvastatin

Fenofibrate is lipid regulating agent. It is used in adjunctive therapy to diet to reduce the elevated LDL-C, Triglycerides in the blood. Structure of fenofibrate is given in Fig No.3.

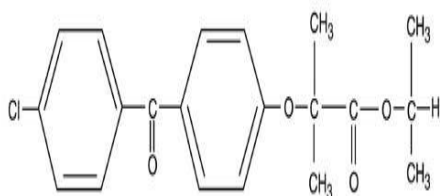


Fig.No.3.Fenofibrate

Literature Survey reveals that there are several analytical methods, existing for individual and combination dosage forms of containing Folic acid, Atorvastatin, fenofibrate by LCMS¹⁻³,

HPLC⁴⁻⁶, TLC⁷⁻⁹ and Spectroscopy¹⁰. There is no reported method of analysis by Ultra Performance Liquid Chromatography for determination of tablets containing Folic acid, Atorvastatin, fenofibrate. Hence, UPLC method in the present work and validated.

Materials and Methods

Folic acid, Atorvastatin, Fenofibrate standards were obtained from SL Drugs and Pharmaceuticals, Hyderabad, India. All reagents used were HPLC grade, Nylon membrane filters of 0.45 μ pore size were used to filter the mobile phase and its components.

Instrumentation

Analysis was carried out in waters acquity with binary UPLC pump equipped with UV detector and empowers 2 software. Separation has been carried out using Agilent SB C8 (2.1 X100) mm, 1.8 μ m column.

Method Development

Various analytical development trials has been performed by using different chemicals and reagents, organic solvents at different pH ranges and strengths in different proportions of buffer and Organic solvents to separate the three peaks with acceptable resolution and with good peak shape. Various stationary phases of multiple makes were used to check the chromatography with acceptable peak shape, tailing factor and plate count for reproducibility at 30° C. Based on the observations and conclusions obtained from the number of chromatographic trials performed on UPLC, a particular set of chromatographic conditions were optimized to be suitable for estimation of the Folic acid, Atorvastatin potassium and Fenofibrate in the tablets. The optimized chromatographic conditions which are found to be suitable for the estimation of the Folic acid, Atorvastatin potassium, Fenofibrate are given below. Table No.1.

Preparation of Diluent

Prepared a mixture of water and Acetonitrile in the ratio of 50:50 (v/v). Mixed well.

Preparation of 0.1% Ortho Phosphoric Acid Buffer

Transferred 1ml of ortho phosphoric acid into 800ml of water and mixed well and volume made up to 1liter with milli Q Water.

Preparation of Mobile Phase

Prepared a mixture of Buffer and Acetonitrile in the ratio of 50:50 (v/v). Mixed well. Sonicated for 10minutes.

Preparations for Methodology

Preparation of Folic acid Standard stock: Weighed and transferred 10.001 mg of folic acid into a 100 ml volume metric flask added 75ml of diluent to it and sonicated for 5 minutes to dissolve the content. Volume made up to the mark with diluent.

Preparation of Atorvastatin Standard stock: Weighed and transferred 10.004 mg of Atorvastatin into a 100 ml volume metric flask added 75ml of diluent to it and sonicated for 5minutes to dissolve the content. Volume made up to the mark with diluent.

Preparation of Fenofibrate Standard stock: Weighed and transferred 100.021 mg of fenofibrate into a 25 ml volume metric flask added 20ml of diluent to it and sonicated for 5minutes to dissolve the content. Volume made up to the mark with diluent.

Preparation of Standard solution: Transferred 5ml from preparation of folic acid standard stock and 10ml from preparation of Atorvastatin standard stock and 4ml from Preparation of Fenofibrate Standard stock into a 100ml volumetric

flask and mixed well. Volume made up to the mark with diluent.

Method Validation

System Suitability

It is assessed by injecting the six replicate into a system. Results are given in Table No.01.

Calibration curve (25-150%)

A linear relationship was evaluated across the range of the analytical procedure and demonstrated directly on the drug substance. The test results were evaluated by calculation of regression line by the method of least square.

The respective each component concentrations were given below.

- A) Folic acid –
1.25,2.50,3.75,5.00,6.25and
7.50ppm
- B) Atorvastatin -
2.50,5.00,7.50,10.00,12.50 and
15.00 ppm
- C) Fenofibrate –
1.00,2.00,3.00,4.00,5.00 and
6.00ppm

All the above prepared solutions of respective component were analysed to calculate the correlations coefficient of the individual components.

Accuracy: Accuracy was performed with 50 to 150% of the test concentration of the respective individual components. The sample solutions prepared with the concentrations are given below for each component.

Folic acid: 2.50, 5.00 and 7.50 ppm

Atorvastatin: 5.00, 10.00 and15.00 ppm

Fenofibrate: 80.00, 160.00, and
240.00ppm

Sample Stock Preparation: Twenty tablets were weighed (each contains 5mg

of folic acid, 10mg of atorvastatin and 80mg of fenofibrate) and calculated average weight. Tablets were crushed into fine powder by using motor and pestle. Weighed one tablet equivalent powder and transferred into 200ml volumetric flask and added 150ml of diluent to it. Allowed to sample extraction by 60mints using sonicator and volume made up to mark with diluent. Filtered through 0.45µm nylon filter.

Sample Preparation

Transferred 10ml of Sample Stock Preparation to 50ml volumetric flask and volume made up to the mark with diluent and injected into system.

Method Precision

The Precision of the method was determined (within day variation) by sample preparation. Calculated % of assay using formula given below.

$$\% \text{ Assay} = \frac{A_t}{A_s} \times \frac{C_s}{C_t} \times P$$

A_t = Area of Sample

A_s = Average area of Standard

C_s = Concentration of Standard

C_t = Concentration of Sample

P = purity of standard

Forced Degradation Study

Acid Degradation: Transferred 10 ml of Sample stock preparation and added 5 ml of 2N Hydrochloric acid solution to round bottom flask and refluxed for 30mins at 60⁰c .Neutralized with 2N NaoH solution. The resultant solution was diluted to obtain test concentration and injected into the system and the chromatograms were recorded to evaluate the degradation of sample.

Alkali Degradation: Transferred 10 ml of Sample stock preparation and added 5 ml of

2N sodium hydroxide solution to round bottom flask and refluxed for 30mins at 60⁰c. Neutralized with 2N hydrochloric acid. The resultant solution was diluted to obtain test concentration with diluent and injected into the system and the chromatograms were recorded to evaluate the degradation of sample.

Oxidation Degradation: Transferred 10 ml of Sample stock preparation and added 5 ml of 30% (v/v) hydrogen peroxide (H₂O₂) to round bottom flask. Refluxed for 30 min at 60⁰c. For UPLC study, the resultant solution was diluted to obtain the test concentration with diluent and injected into the system and the chromatograms were recorded to evaluate the degradation.

Dry Heat Degradation: Transferred 10ml of Sample stock preparation into 50ml volumetric flask separately and placed in oven at 70°C for 1 hour to study dry heat degradation. For UPLC study, the resultant solution was diluted with diluent up to mark and injected into the system and the chromatograms were recorded to evaluate the degradation of the sample.

Photolytic Degradation: The photochemical stability of the drug was also studied by exposing the sample Powder and sample placed under UV Light by keeping the beaker in photo stability chamber For 1day or 200 Watt hours/m². The resultant solution was diluted to obtain test concentration and injected into the system and the chromatograms were recorded to evaluate the degradation of sample.

RESULTS AND DISCUSSION

Method Optimization:

The developed method was optimized after many trials. The optimized method developed on SB C8 (2.1 x100) mm, 1.8µm as stationary phase. Using

0.1%ortho phosphoric acid buffer and Acetonitrile in the ratio of (50:50v/v) as mobile phase. The column temperature was maintained constantly at 30°C. Mobile

phase pumped with a flow rate of 0.3ml/minute and injection volume is 1.00µL.

Table No.1: Chromatographic conditions

Chromatographic conditions	
Column	Agilent SB C8(2.1 X100mm),1.8µm
Flow rate	0.3ml/mint
Column Temperature	30°C
Injection Volume	1.0µL
Detector wavelength	210 nm
Separation Mode	Isocratic
Run Time	04minutes

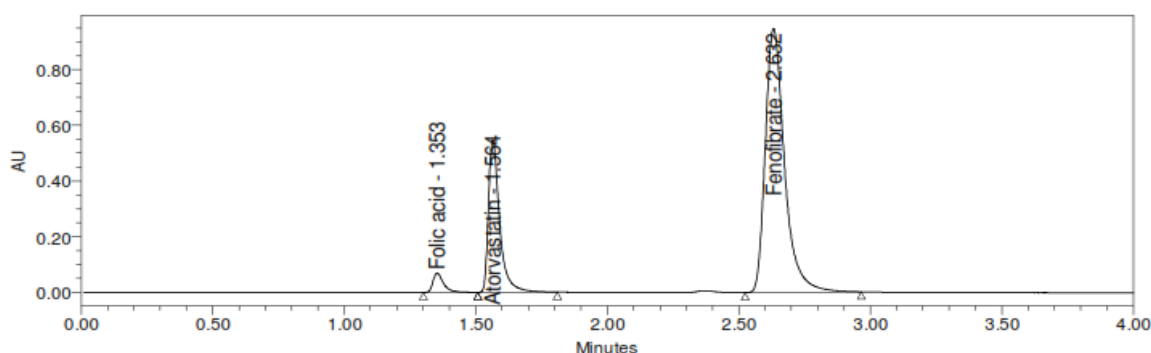


Fig 4: System Suitability Chromatogram

Table 02: System Suitability

Name of the Component	Retention Time	Area	USP Plate Count	USP Tailing Factor	USP Resolution
Folic Acid	1.35	188545	6085	1.6	
Atorvastatin	1.56	403346	7335	1.7	2.9
Fenofibrate	2.63	4774058	6905	1.4	10

Result Table No: 03

Linearity		
Name of the Analyte	Regression Equation	Regression coefficient
Folic acid	Y =37735 +3988	0.9988
Atorvastatin	Y= 40204 x +6488	0.9991
Fenofibrate	Y= 30060 x + 98203	0.9989

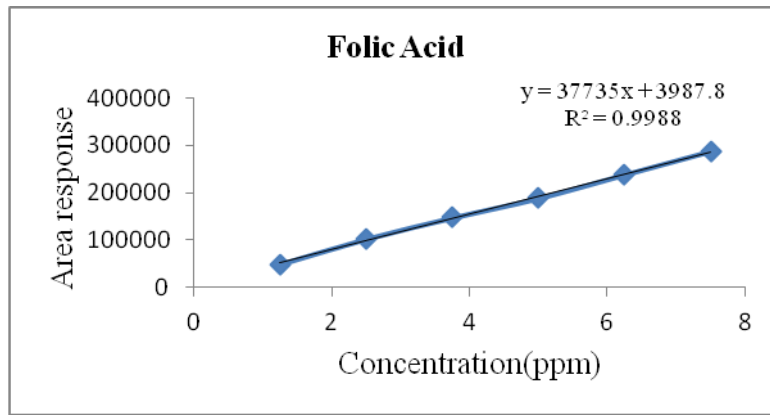


Fig No.5a Folic acid linearity graph

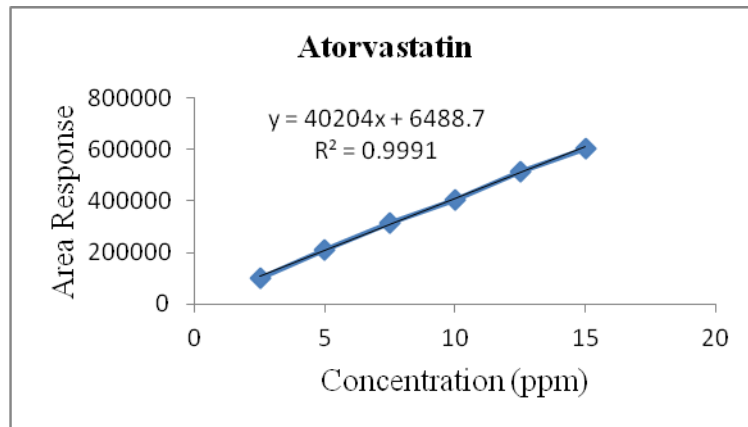


Fig No.5b Atorvastatin linearity graph

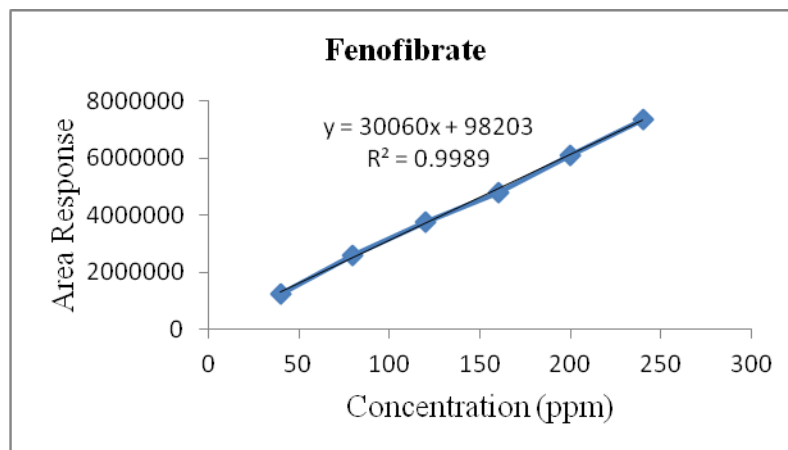


Fig No.5c Fenofibrate linearity graph

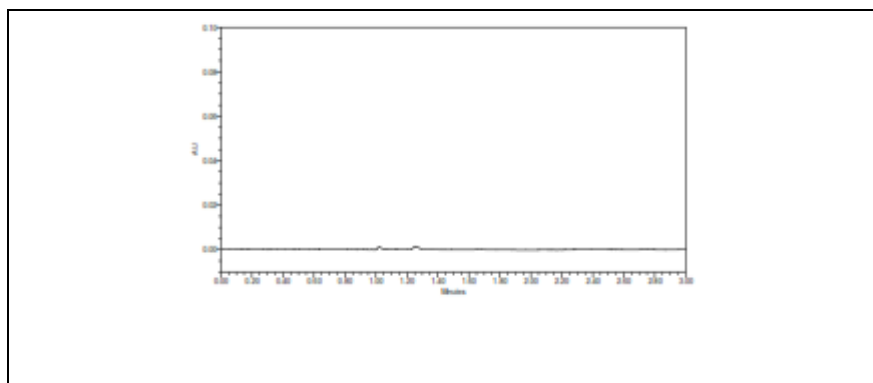


Fig No.06a-.Blank Chromatogram

Table No.04

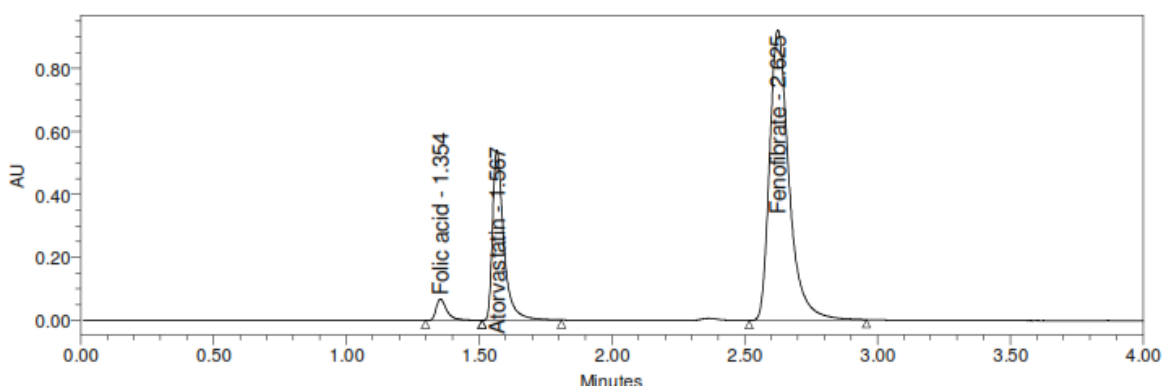


Fig No.6b typical chromatogram for specificity

Result Table No.04

Precision		
Name of the Analyte	Method Precision	Intermediate Precision
Folic acid hydrochloride	0.5% RSD	0.7%RSD
Atorvastatin	1.0%RSD	1.1%RSD
Fenofibrate	0.6%RSD	0.5%RSD

Table No.05

Level	Folic Acid			Atorvastatin			Fenofibrate		
	Added(ppm)	Found	% Mean Recovery	Added	Found	% Mean Recovery	Added	Found	% Mean Recovery
50	2.50	2.52	100.84	5.00	4.95	99.57	80	79.51	99.24
50	2.50	2.53		5.00	5.00		80	79.40	
50	2.50	2.52		5.00	4.99		80	79.27	
100	5.00	4.84	98.67	10.00	10.01	99.97	160	159.66	99.36
100	5.00	5.00		10.00	100.1		160	158.74	
100	5.00	4.96		10.00	9.98		160	159.51	
150	7.50	7.46	99.69	15.00	14.97	99.8	240	240.76	100.18
150	7.50	7.48		15.00	14.97		240	240.45	
150	7.50	7.49		15.00	14.97		240	240.10	

Table No.06

Folic Acid	Atorvastatin	Fenofibrate
Flow Rate (0.27ml/mt)		
196960	402749	4680365
Flow Rate(0.33ml/mt)		
168228	391465	3967970
55:45(Buffer:Acetonitrile)		
197774	403499	4670837
45:55(Buffer:Acetonitrile)		
196167	405453	4682828
Temperature 25°C		
196960	406082	4680365
Temperature35°C		
195873	405541	4666581

Table No.07

Condition	Folic acid	Atorvastatin	Fenofibrate
%Degradation			
Acid	3.07	4..05	3.52
Base	1.37	2.29	1.21
Oxidative	0.43	1.29	1.14
Thermal	0.05	0.44	0.70
Photolytic	0.51	0.54	3.99

The chromatographic peaks were detected and measured at 215 nm. The retention times of Folic acid, atorvastatin and Fenofibrate were found to be 1.0, 1.66 and 2.0.

Method Validation

System suitability: All system suitability parameters were passed which include the USP plate count, USP tailing USP resolution for folic acid, atorvastatin and fenofibrate respectively. Fig No.04 and Table No.02.

Linearity: The best fit line was obtained with regression coefficient between the peak response vs concentration. Results

are given below .Table No.03 and Figure No.5a, 5b and 5c.

Specificity: It was evaluated by injecting blank and placebo along with drug product, no interference was found at the components respective retention timings. Chromatogram depicted below Fig No.06a and 6b

Method Precision: The Precision of the method was determined (within day variation) by injecting Folic acid hydrochloride, Atorvastatin, Fenofibrate with sample solution 6 times respectively. Method precision was expressed in terms of % RSD. Results are given in table no.04.

Accuracy: Prepared accuracy at 3 levels in triplicate at 50% 100% and 150% with matrix and achieved satisfactory results and at each level of recovery was calculated. Results are given below table No.05.

Robustness: Measure of the method capability to remain unaffected by small variations such as flow rate, mobile phase, temperature. Results are given below, Table No.06.

Forced Degradation: It was evaluated by using the different stress condition to assess the degradation. Calculated their degradation in terms of %. Results are given below Table. No.07.

CONCLUSION

The developed method is new and simple which met the within acceptance criteria as system suitability, tailing factor, resolution among the individual components and reproducibility. Method is specific, precise, robustness and reliability. Drugs are separated within in short span of time. The method is economically also very less over than conventional method. So that based on obtained results from the method and can be use in routine analysis of quality control.

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