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NOVEL RP HPLC METHOD FOR THE ASSAY OF PRAVASTATIN AND FENOFIBRATE IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, fast and precise RP-HPLC method is described for simultaneous determination of Pravastatin and Fenofibrate in tablet dosage form. Chromatographic separation of these two drugs was achieved on Inertsil ODS C18 (250 x 4.6 mm, 5 µm) as stationary phase with a mobile phase Ammonium acetate buffer (pH 2 adjusted with OPA): Acetonitrile: Methanol (40:40:20% v/v) at a flow rate of 1 ml/min and PDA detection at 240 nm. The method was carried out at ambient temperature. The retention times of Pravastatin and Fenofibrate were found to be 2.2±0.1 min, 3.7±0.1min respectively. The proposed method was validated for system suitability, linearity, accuracy, precision, LOD, LOQ and robustness. The calibration curves were linear in the concentration range of 50% to 150% of the working concentration (r2 = 0.999) for both the drugs in binary mixture. The LOD was found to be 0.25 µg/ml & 4.60 µg/ml and LOQ was found to be 0.76 µg/ml & 13.95 µg/ml for Pravastatin and Fenofibrate respectively. Hence, the proposed RP-HPLC method of analysis can be used in quality control departments with respect to routine analysis of tablets containing Pravastatin and Fenofibrate.

INTRODUCTION:

Pravastatin is (3*R*,5*R*)-3,5-dihydroxy-7-[(1*R*,2*S*,6*S*,8*R*,8a*R*)-6-hydroxy -2 - methyl - 8-{[(2*S*) -2 -methylbutanoyl]oxy}-1, 2 ,6 ,7, 8, 8a-hexahydronaphthalen-1-yl]-heptanoic acid, is used in the treatment of hyperlipidemia. Fenofibrate is 2- [4- (4- chlorobenzoyl)phenoxy]-2-methyl-propanoic acid, 1-methylethyl ester. It is used in the treatment of hyperlipidaemea. Pharmaceutical preparation contains 20 mg Pravastatin 160 mg Fenofibrate that is available in the market with the trade name Pravafenix as hard capsules. Pravastatin and Fenofibrate are official in BP and USP[1, 2.]

The literature survey revealed there are no analytical works available for simultaneous determination of above specified drugs in pharmaceutical preparations by RP-HPLC. Hence it is a novel RP HPLC method for simultaneous determination of Pravastatin and Fenofibrate from their pharmaceutical dosage form. The method described is simple, fast, precise, accurate for simultaneous determination of Pravastatin and Finofibrate from pharmaceutical preparation, and validated as per ICH guidelines[3].

MATERIALS AND METHODS:

Reference Standards of Pravastatin and Fenofibrate were obtained with certificate of analysis. Pravafenix (Ranbaxy Laboratories) has been taken for study which is purchased from local market. Ammonium acetate buffer, Ortho Phosphoric Acid, Acetonitrile, Methanol and HPLC grade water were used of analytical grade from Merck. Mobile phase is used as diluent.

Standard Stock Solution:

10 mg of Pravastatin working standard is accurately weighed and transferred carefully into a 50 ml volumetric flask, 5 ml of methanol is added to dissolve and then 25 ml of diluent is added and sonicated and made up to the volume with same solvent. 80 mg of Fenofibrate working standard and is accurately weighed and transferred carefully into a 50 ml volumetric flask, 5 ml of methanol is added to dissolve and then 25ml of diluent is added and sonicated and made up to the volume with same solvent.

Working Standard Solution:

5ml from each standard solution of Pravastatin and Fenofibrate is transferred in to a 50 ml of volumetric flask, made up to 50 ml with diluent and filtered.

Sample Preparation:

20 tablets were weighed accurately and powdered. Powder which is equivalent to10 mg of Pravastatin and 80 mg Fenofibrate is weighed and transferred into 50 ml volumetric flak, 10 ml of methanol. Sonicated for few minutes and cooled to room temperature. Then it is diluted up to 50 ml with mobile phase and filtered through Whatmann No.1 filter paper. From the resulted solution 5 ml has been transferred in 50 ml volumetric flask and made up with diluent.

Preparation of buffer:

25 mg of ammonium sulfate is dissolved in 235 ml of water (solution A). 105 ml of 2 N sodium hydroxide solution and 135 ml of 2 N acetic acid were mixed (this is solution B). Add 25 ml of solution A to solution B and mixed well. Using a p^H meter, the p^H of this solution is adjusted to 2 using OPA.

Chromatographic conditions:

The criteria employed for selecting the mobile phase for the analysis of the drugs were cost involved, time required for the analysis and better separation of drugs. Chromatographic separation was performed on reverse phase Inertsil ODS C_{18} (250 x 4.6 mm, 5 µm) as stationary phase with a mobile phase of Ammonium acetate buffer (p^H 2 adjusted with OPA): Acetonitrile : Methanol (40:40:20% v/v) at a flow rate of 1 ml/min and PDA detection at 240 nm. The method was carried out at ambient temperature. About 20 µl of standard and sample solutions were injected for simultaneous determination of Pravastatin and Fenofibrate

Method Development:

Different chromatographic conditions were tried for separation and resolution. Inertsil ODS C_{18} column was found satisfactory. Peak purity of Pravastatin and Fenofibrate was checked using PDA detector and 240 nm was considered satisfactory for detecting both the drugs with adequate sensitivity. The optimized chromatographic conditions are presented in Table No. 1. A typical RP-HPLC chromatogram for simultaneous determination of Pravastatin and Fenofibrate is shown in Fig. No.1.

Validation of the method:

The developed RP-HPLC method was validated for parameters like system suitability, accuracy, precision, linearity, Limit of Detection, Limit of Quantitation and robustness. A sample from the placebo was prepared in the same way as the sample under the conditions prescribed in the analytical procedure and the area of placebo sample was measured. There observed no peaks in the retention times of analyte peaks. Then a blank solution was prepared without the analytes and observed for any interfering peaks at the retention times of analytes. There observed no peaks. The chromatograms of blank, placebo and standards are in Fig. No. 2-3.

System suitability

System suitability tests are used to verify the reproducibility of the equipment is adequate for the analysis to be carried out. The test was carried out by injecting 20 µl standard solutions in six replicates. Theoretical plates, resolution, asymmetry were determined and found to be satisfactory. The results are presented in Table No. 2.

Accuracy:

Accuracy of the method was determined by recovery studies which were carried out by applying the standard addition method. A known quantity of drug substance corresponding to 50%, 100%, and 150% of the label claim of drug

were added, to determine if there are positive or negative interferences from excipients present in the formulation. Each set of addition were repeated three times. The accuracy was expressed as the percentage of analytes recovered by the assay. The results were summarized in Table No. 3-4 which has shown that the recoveries of the drug from a series of spiked concentrations are in the range 99.68±1. The results indicate the method was accurate.

Method precision

The method precision was determined from results of six independent determinations at 100% of the test concentrations of Pravastatin and Fenofibrate in the product. The % RSD was found to be within the limits. The results obtained were tabulated in Table No.5.

Intermediate Precision:

The % relative standard deviations of Pravastatin and Fenofibrate for intermediate precision are found to be within the limits. Hence indicate a good degree of precision. The results are tabulated in Table No. 6.

System precision:

The standard and sample solutions are prepared by analyst-1 and analyst-2 are injected in different HPLC systems in different laboratories on different days. The results were tabulated in the Table No.7.

Linearity

Linearity was evaluated by analysis of standard solutions of Pravastatin and Fenofibrate of five different concentrations. Linearity was assessed by performing single measurement at several analyte concentrations. Varying quantities of the mixed standard stock solutions was diluted with the mobile phase to give concentrations of 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml and 30 µg/ml of Pravastatin and 80 µg/ml, 120 µg/ml, 160 µg/ml, 200 µg/ml and 240 µg/ml of Fenofibrate. The peak area ratio and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. The linearity graph was plotted from Fig. No.4-5.The regression data obtained for Pravastatin and Fenofibrate is represented in Table No. 8. The result shown that within the concentration range mentioned above, there was an excellent correlation between peak area and concentration.

Limit of Detection and Limit of Quantitation:

The limit of detection (LOD) and limit of quantitation (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively. The LOD and LOQ of Pravastatin and Finofibrate were experimentally determined by injecting six injections of each drug. The LOD of Pravastatin and Fenofibrate was found to be 0.25 µg /ml & 4.60 µg /ml respectively. The LOQ of Pravastatin and Fenofibrate was found to be 0.76 µg /ml & 13.95 µg / ml respectively.

Robustness:

Small changes in flow rate and wavelength are performed for the robustness of the method. The chromatograms of drug solution were recorded with different flow rates such as 0.8 ml/min, 1.0 ml/min and 1.2 ml / min. At the flow rate of 1.0 ml / min, the peaks were sharp with good resolution, apart from above said flow rate, rest of the flow rates were found to be not satisfactory. But passed all system suitability parameters indicating the method is robust. The results are presented in Table No. 9. The chromatograms of drug solution were recorded with different wave lengths such as 238 nm,240 nm and 242 nm. At the 240 nm the peaks were sharp with good resolution, apart from above said temperature, rests of the wave lengths were found to be not satisfactory, although passed all system suitability parameters indicating the method is robust. The results are presented in Table No.10.

CONCLUSION

A new RP-HPLC method was developed and validated as per ICH Q2 (R1) guidelines for the simultaneous estimation of Pravastatin and Fenofibrate in pharmaceutical dosage form. The results of the study indicate that the proposed method of analysis can be used in quality control departments with respect to routine analysis for the assay of Pravastatin and Fenofibrate in bulk and pharmaceutical dosage form.

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Fig No. 1: Chromatogram of Pravastatin and Fenofibrate

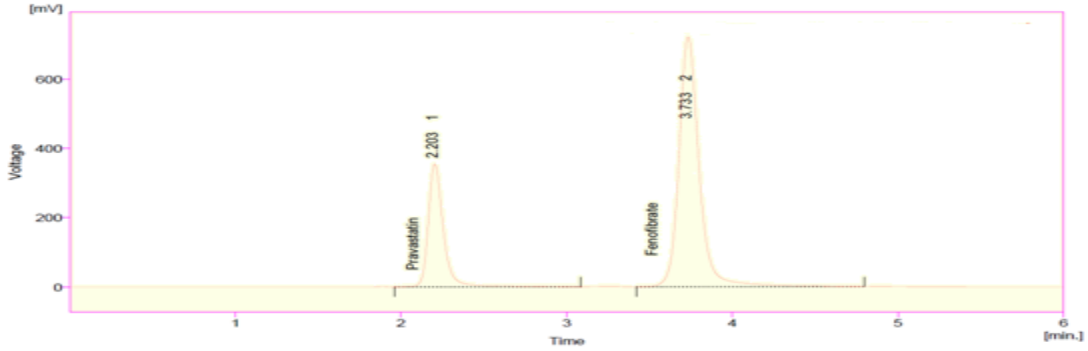

Fig No. 2: Chromatogram of blank

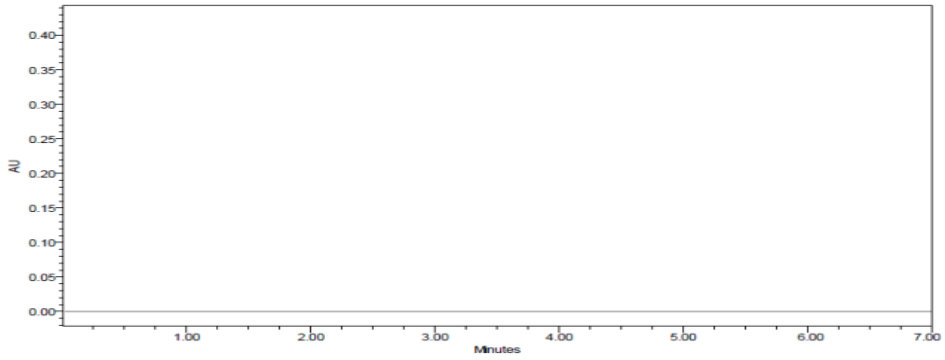

Fig No. 3: Chromatogram of placebo

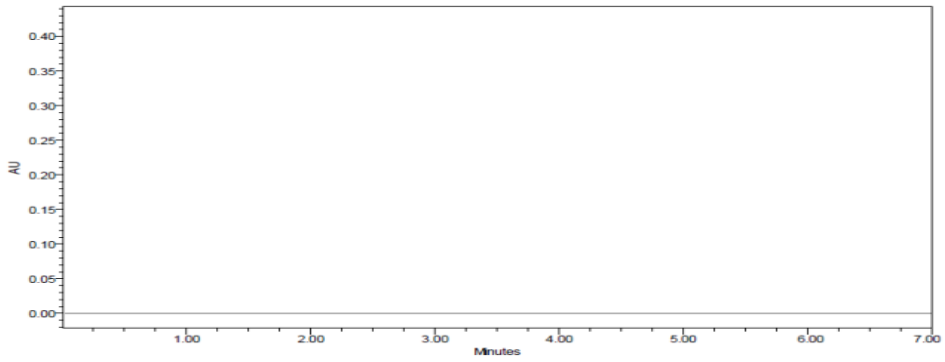

Fig No. 4: Linearity chart of Pravastatin

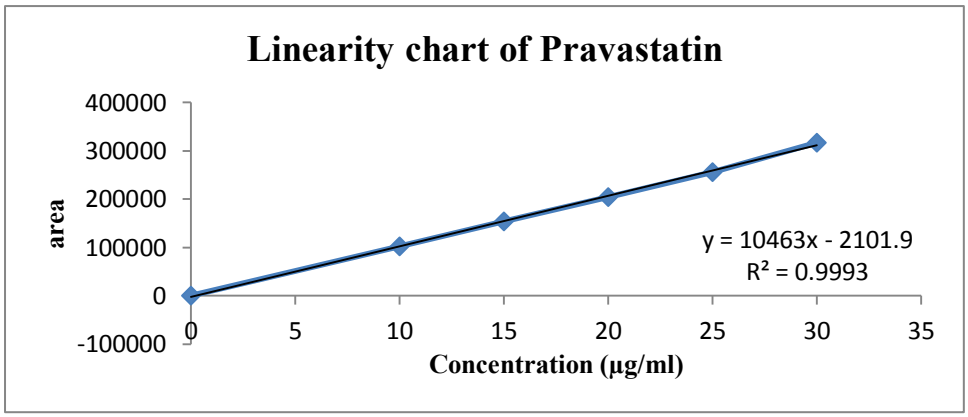

Fig No. 5: Linearity chart of Fenofibrate

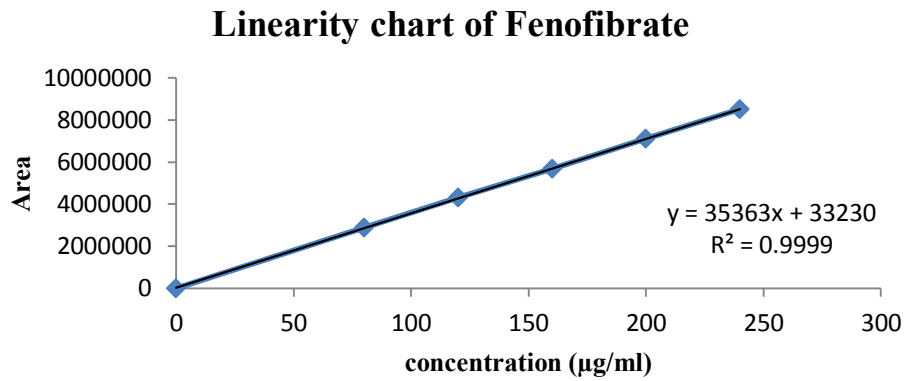

Table No.1: Optimized chromatographic conditions

Parameters	Method
Stationary Phase (column)	Intersil ODS C18 (250x4.6 mm, 5 µm)
Mobile Phase	Ammonium acetate buffer (pH 2):Acetonitrile: methanol (40:40:20)
Flow Rate (ml/min)	1.0 ml/min
Runtime (min)	6 min
Column Temparature (^{o}C)	Ambient
Injection volume (µl)	20 µl
Detection wavelength (nm)	240 nm
Retension times (min)	Pravastatin:2.2±0.1 Fenofibrate: 3.7±0.1

Table No.2: System suitability parameters for Pravastatin and Fenofibrate

Analytes	Resolution	Tailing Factor	Retention times	Theoretical plates
Pravastatin	-	1.409	2.21	33506
Fenofibrate	8.422	1.219	3.73	50944

Table No.3: Data of Accuracy for Pravastatin

% of Spiked level	Amount added (µg)	Amount recovered (µg)	% Recovery	% Mean Recovery
50%	10	10.14	101.4	100.43
50%	10	10.03	100.3	
50%	10	9.96	99.6	
100%	20	20.08	100.4	100.65
100%	20	19.97	99.85	
100%	20	20.36	101.8	
150%	30	30.07	98.89	99.68
150%	30	30.11	100.30	
150%	30	29.96	99.86	

Table No.4: Data of Accuracy for Fenofibrate

% of Spiked level	Amount added (µg)	Amount recovered (µg)	% Recovery	% Mean Recovery
50%	80	80.14	100.17	100.05
50%	80	80.03	100.03	
50%	80	79.96	99.95	
100%	160	159.88	99.92	99.97
100%	160	159.97	99.98	
100%	160	160.06	100.03	
150%	240	240.07	100.02	99.99
150%	240	239.91	99.96	
150%	240	239.99	99.99	

	Injection	Area of Pravastatin	Area of Fenofibrate
At 100% concentration	1	2051034	5647870
	2	2003811	5639718
	3	2062991	5692951
	4	2052703	5602739
	5	2039124	5765096
	6	2058026	5740309
Statistical Analysis	Mean	2044615	5681439
	S.D	21537.01	62700.01
	% RSD	1.05	1.10

Table No. 5: Data for Method precision for Pravastatin and Fenofibrate

Table No. 6: Data of Intermediate precision (Analyst I & II) for Pravastatin and Fenofibrate

Analyst I			Analyst II	
Injection at 100% concentration	Area of Pravastatin	Area of Fenofibrate	Area of Pravastatin	Area of Fenofibrate
1.	2041034	5657876	2125142	5587452
2	2001034	5607846	2091202	5497618
3.	2011041	5617816	2145145	5517204
4.	2021024	5607866	2125142	5527254
5.	2031201	5537609	2105140	560725
6.	2091201	5507609	2105105	550725
Mean	2032756	5589437	2116146	5540671
S.D	31944.30	55767.02	19329.01	45437.21
%RSD	1.57	1.00	0.91	0.82

Table No. 7: Data of System Precision (Laboratory I &II) for Pravastatin and Fenofibrate

Laboratory-I			Laboratory-II	
Injection at 100% concentration	Area of Pravastatin	Area of Fenofibrate	Area of Pravastatin	Area of Fenofibrate
1.	2125142	5587452	2051034	5647870
2	2091202	5497618	2003811	5639718
3.	2145145	5517204	2062991	5692951
4.	2125142	5527254	2052703	5602739
5.	2105140	5630725	2039124	5765096
6.	2105105	5540725	2058026	5740309
MEAN	2116146	5540671	2044615	5681439
S.D	19329.01	45437.21	21537.01	62700.01
%RSD	0.91	0.82	1.05	1.10

Table No. 8: Data of Linearity for Pravastatin and Fenofibrate

S. no.	Pravastatin		Fenofibrate	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
	10	102216	80	2891262
	15	153877	128	4311277
	20	204392	160	5682340
	25	256498	192	7103626
	30	316677	240	8501210
Slope	10463		35363	
y-intercept	2101		33230	
Correlation coefficient	0.999		0.999	

Table No. 9: Data for Robustness studies (for variant flow rate)

Analyte	Flow rate (ml/min)	Plate count	Tailing
Pravastatin	0.8	30443	1.223
	1.0	33506	1.409
	1.2	29784	1.161
Fenofibrate	0.8	45673	1.232
	1.0	50944	1.219
	1.2	49006	1.145

Table No. 10: Data for Robustness studies (for variant wave length)

Analyte	Wave length (nm)	Plate count	Tailing
Pravastatin	238	29872	1.113
	240	33506	1.409
	242	28874	1.341
Fenofibrate	238	43952	1.552
	240	50944	1.219
	242	46733	1.157

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