Journal of Advanced Zoology
ISSN: 0253-7214
Volume 45 Issue 1 Year 2024 Page 420-434

Development and Validation of Analytical methods for estimation of Simvastatin and Fenofibrate

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Article History

Received: 10 Nov 2023
Revised: 25 Nov 2023
Accepted: 20 Dec 2023

Abstract

The present work describes Stability indicating RP-HPLC and First order derivative UV spectrophotometric method for the quantitative determination of Simvastatin and Fenofibrate. Materials and methods: The parameters Specificity, linearity, accuracy, precision, detection limit, quantitation limit, Robustness and system suitability tests were studied and their results were compiled to ICH guideline Q2 (R2). Chromatography was carried out by reverse phase technique on an RP-18 with mobile phase composed of Acetonitrile: Water (90:10; %v/v) adjusted to pH 3.3 with 10% orthophosphoric acid) with flow rate 1 ml/min. Both drugs were eluted, isocratically using detection wavelength 230 nm. Methanol was used as a solvent, the spectrum was recorded between 200-400 nm wavelengths, and all the zero-order spectrum (D_0) were converted to first-order derivative spectrum (D_1) using delta lambda 2.0 and scaling factor 4. 240 nm (zero crossing point of Fenofibrate) and 306 nm (zero crossing point of Simvastatin) were used for determination of Simvastatin and Fenofibrate, respectively. Regression analysis of UV-Spectrophotometric method showed good linearity r^2 = 0.9991 at 240 nm of Simvastatin 1-5 µg/ml and r^2 = 0.9998 at 306 nm of Fenofibrate 10-50 µg/ml. For proposed methods, the linearity for both methods were obtained in the concentration range of 1-5 µg/ml for Simvastatin and 10-50 µg/ml for Fenofibrate. Statistical analysis by student's t-test showed no significance difference between the results obtained by these two methods. Results: The suitability of method for the quantitative determination of Simvastatin and Fenofibrate was proved by validation. Conclusion: The proposed methods and its results had been successfully applied and validated statistically to the simultaneous estimation of Simvastatin and Fenofibrate in their combination for quality analysis.

Keywords: Simvastatin, Fenofibrate, Hypercholesterolemia, RP-HPLC method, First-order derivative UV method.

INTRODUCTION

Simvastatin, (1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate[1], is in a Group of drugs called HMG CoA Reductase Inhibitors, or "statins." It reduces levels of Bad Cholesterol (Low-Density Lipoprotein, or LDL) and triglycerides in the blood, while increasing levels of "Good" Cholesterol (High-density Lipoprotein, or HDL)[2]. Fenofibrate, propan-2-yl 2-[4-(4-chlorobenzoyl) phenoxy]-2-methylpropanoate[1], is a fibric acid derivative like Clofibrate and Gemfibrozil. Fenofibrate is used to treat Primary Hypercholesterolemia, mixed Dyslipidaemia, severe Hypertriglyceridemia[3]. Individually, Simvastatin and Fenofibrate are available in different dosage forms in market. Number of clinical trials on Simvastatin and Fenofibrate in combination has been performed using by Researchers. In view of Clinical Trials, Combination of Simvastatin and Fenofibrate was under study clinical trial phase and was -proved that the combination is effective significantly more improved Endothelium-Dependent vasodilation.[4-5] From the Exhaustive literature survey, Analysis of Simvastatin and Fenofibrate by various methods like Spectroscopic methods viz. UV and Mass Spectroscopy; and Chromatographic methods viz. HPTLC[6], Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)[7-8], Simvastatin alone[9-15], Fenofibrate alone[16-23], Simvastatin and Ezetimibe[24-25], Simvastatin and Sitagliptin Phosphate[26-27], Fenofibrate and Rosuvastatin Calcium[28-30], Fenofibrate and Atorvastatin[31-32], Fenofibrate and Metformin Hydrochloride[33-34], Fenofibrate and Pravastatin[35-36] and many more. Since no method has been develop and validated for simultaneous estimation of Simvastatin and Fenofibrate in combination. Hence, the objectives of the present work were to develop and validate Stability indicating RP-HPLC and First-order derivative UV Spectrophotometric method for simultaneous estimation of Simvastatin and Fenofibrate in combination.

MATERIALS AND METHODS

Chemicals and Reagents

The bulk drug, Simvastatin and Fenofibrate were procured as gift sample from M/S Biocon Pvt. Ltd., Ahmedabad and Torrent Pharmaceuticals Ltd., Ahmedabad, respectively. Methanol, Acetone and Water used of HPLC grade were procured from Finar chemicals, Ahmedabad. Potassium dihydrogen phosphate and ortho phosphoric acid, 75 % (AR Grade) were purchased from Astron Chemicals Ltd., India. All solutions were prepared fresh on daily basis.

Instrumentation and Analytical condition

The HPLC method was performed on Systronic RP-HPLC (LC-138), UV Detector SPD-20 A, Rheodyne injector fitted with a 20 µl loop and used Clarify® software for determination. The method was conducted using Reverse phase techniques. Both drugs were eluted isocratically using Acetonitrile: Water (pH 3.3 adjusted with 10 % Ortho Phosphoric Acid) (90:10; v/v) with flow rate 1 ml/min. The detection wavelength of UV-vis Detector was set to 230 nm. All solutions with mobile phase were prepared daily, which were filtered by 0.45 µm membrane filter (Millipore) and sonicated with Sonicator (Equitron, India) before use. A Kromstar® C_{18} (250 × 4.6 mm, 5 µm) Column and Systronics® pH meter was used. The HPLC system was operated at room temperature (25 ± 1°C).

UV Spectrophotometric method was performed on Shimadzu UV Visible double beam spectrophotometer (Model-1900) and using 1.0 cm quartz cells. UV Probe software was used for all absorbance measurements. All weighing were done on Digital Analytical balance (Wensar Dab 13-220).

Preparation of Standard Solution

Accurately weighed 10 mg of Simvastatin and 10 mg of Fenofibrate standard were transferred to 100 ml volumetric flask and dissolved in 100 ml methanol. The flasks were shaken and volume was made up to the mark with Methanol to give solution containing 100 µg/ml of Simvastatin and 100 µg/ml of Fenofibrate. From this solution, Simvastatin was pipetted out as aliquots 0.1, 0.2, 0.3, 0.4 and 0.5 ml and Fenofibrate was pipetted out as aliquots 1, 2, 3, 4 and 5 ml of the stock solution were further diluted to 10 ml volumetric flasks individually with methanol to get concentrations 1, 2, 3, 4 and 5 µg/ml for Simvastatin and 10, 20, 30, 40 and 50 µg/ml for Fenofibrate.

Preparation of Sample solution

Accurately weighed equivalently weight of Simvastatin (1 mg) and Fenofibrate (10 mg) which transferred in 100 ml volumetric flask and make up half mark with Methanol. This solution was sonicated till the drug

dissolves and was made upto mark with methanol. This solution was filtered through Whatmann filter paper. The concentration of Simvastatin was 10 µg/ml and Fenofibrate was 100 µg/ml. From above mixture solutions, 2 ml transferred in to a 10 ml volumetric flask and the volume was adjusted up to the mark with mobile phase to make final concentration of Simvastatin 2 µg/ml and Fenofibrate 20 µg/ml.

Selection of wavelength detection

Simvastatin (2 µg/ml) and Fenofibrate (20 µg/ml) were used for the detection of wavelength.

RP-HPLC Method

The sensitivity of RP-HPLC method that uses UV detection depends upon proper selection of detection wavelength. Simvastatin and Fenofibrate were observed good peak height resolution and shape at 230 nm. Hence, wavelength of 230 nm was selected for further study (Figure 1).

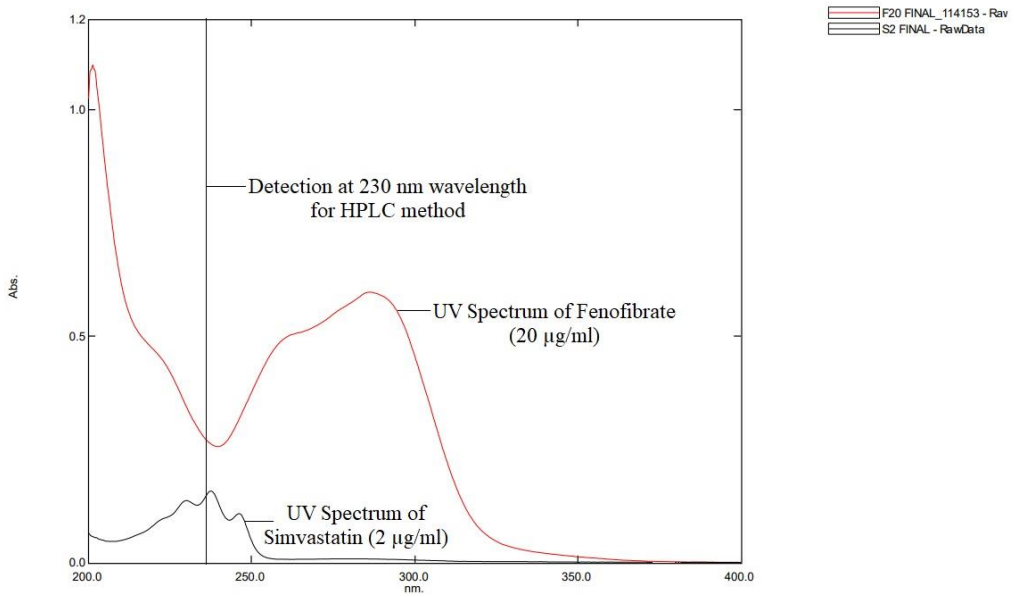

Figure 1: Overlay UV Spectrum of Simvastatin (2 µg/ml) and Fenofibrate (20 µg/ml) in Methanol (Zero order) showing 230 nm selected wavelength for HPLC

UV method (First order derivative) method

The solutions were scanned and their spectra were recorded in the range of 200-400 nm against Methanol as a reagent blank. Absorbance maximum (λmax) were obtained at their λmax 237 nm and 286 nm for Simvastatin and Fenofibrate, respectively. The overlain UV spectrum of Simvastatin (2 µg/ml) and Fenofibrate (20 µg/ml) in Methanol (Zero order D_0) which showed its maximum wavelength represented in figure 2 and 3.

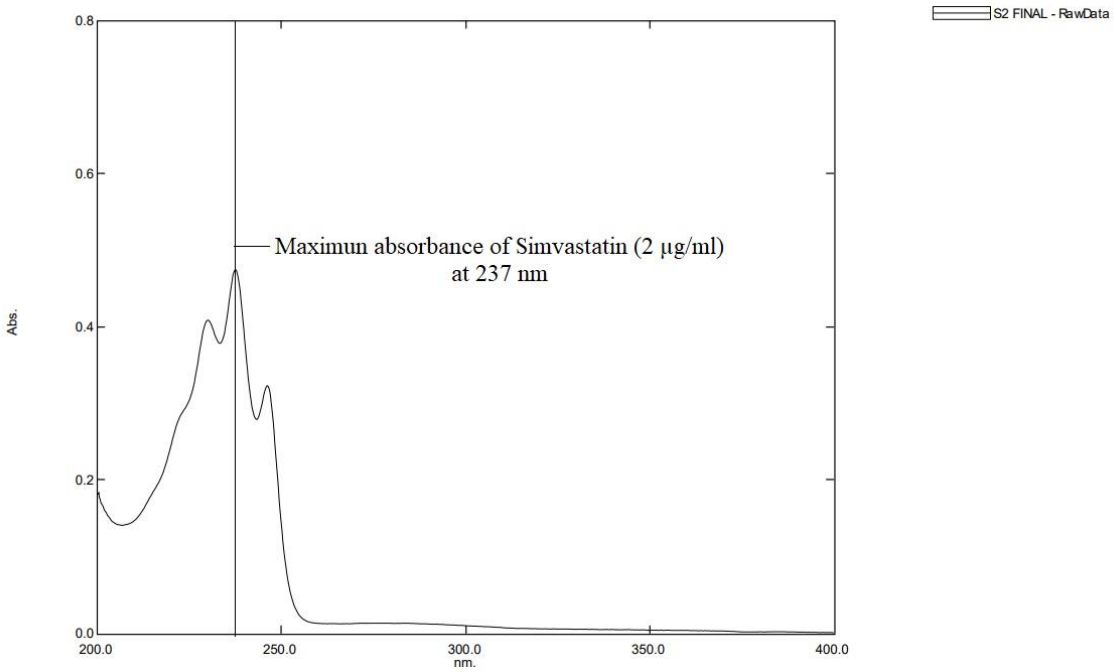

Figure 2: UV Spectrum of Simvastatin (2 µg/ml) at 237 nm

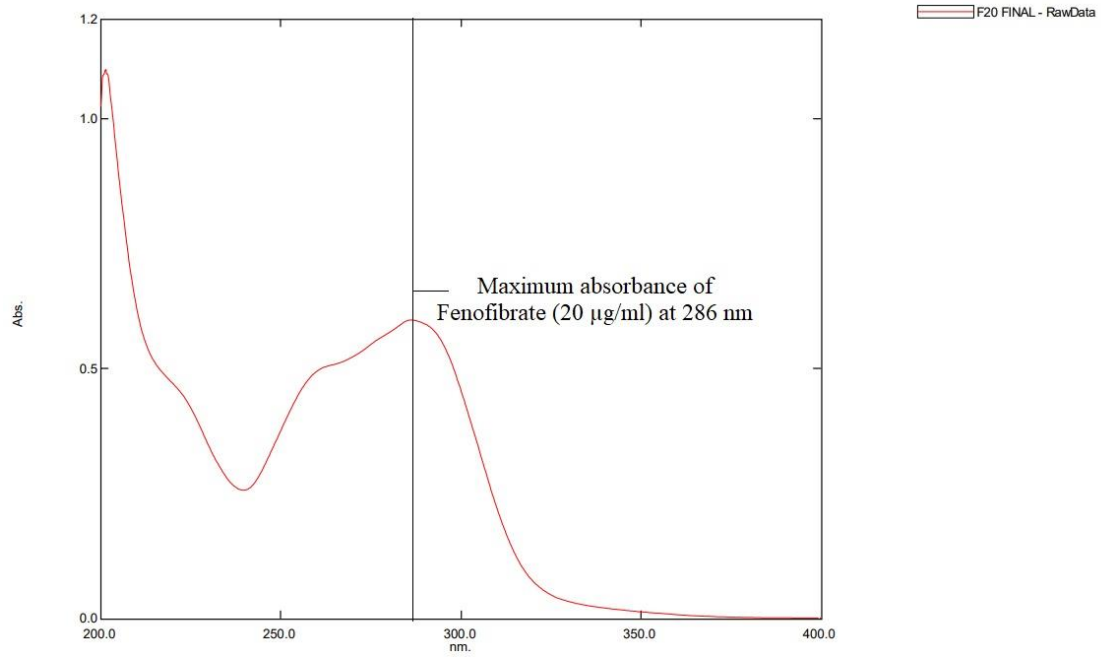

Figure 3: UV Spectrum of Fenofibrate (20 µg/ml) at 286 nm

All zero-order spectrum (D_0) were converted to first derivative spectrum (D_1) using delta lambda 2.0 and scaling factor 4. The overlain first derivative spectrums of Simvastatin and Fenofibrate at different concentrations were recorded. The zero-crossing point (ZCP) of Fenofibrate and Simvastatin were found to be 240 nm and 306 nm, respectively. The overlain UV spectra of Simvastatin (2 µg/ml) and Fenofibrate (20 µg/ml) in Methanol (First order D_1) represented in figure 4.

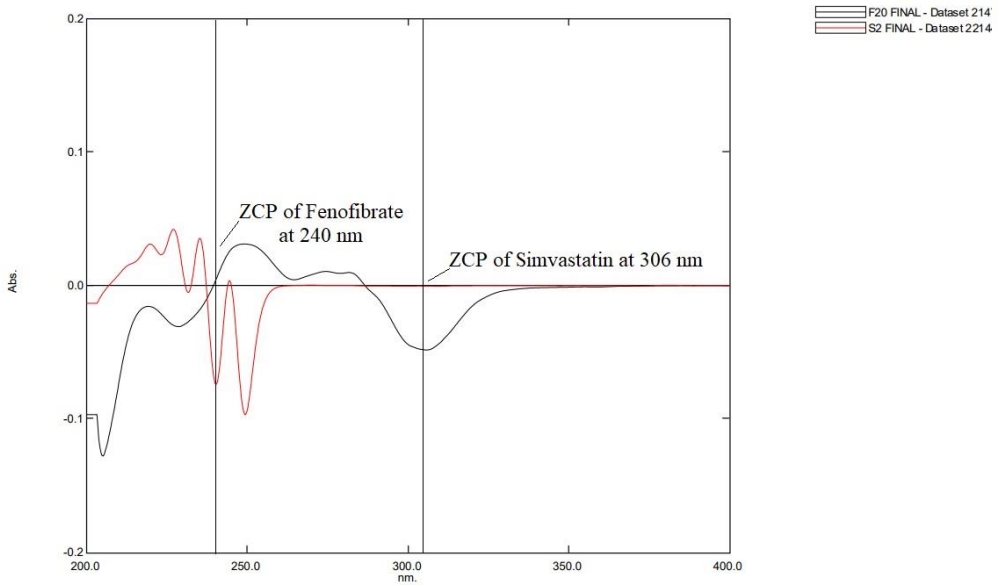

Figure 4: Overlain UV Spectra of Simvastatin (2 µg/ml) and Fenofibrate (20 µg/ml) in Methanol (First order)

Method Validation

The Methods were validated as per ICH guideline Q2(R2)[23]. The proposed method has been extensively validated in terms of Specificity, Linearity and range, Accuracy, Precision, Detection limit, Quantification limit, Robustness and System suitability tests.

Specificity

Sample solutions (Simvastatin 2 µg/ml and Fenofibrate 20 µg/ml) were performed to verify degradation and interferences (Figure 5). None interference was found with the Chromatogram of Simvastatin, Fenofibrate and blank resulted in method was Specific.

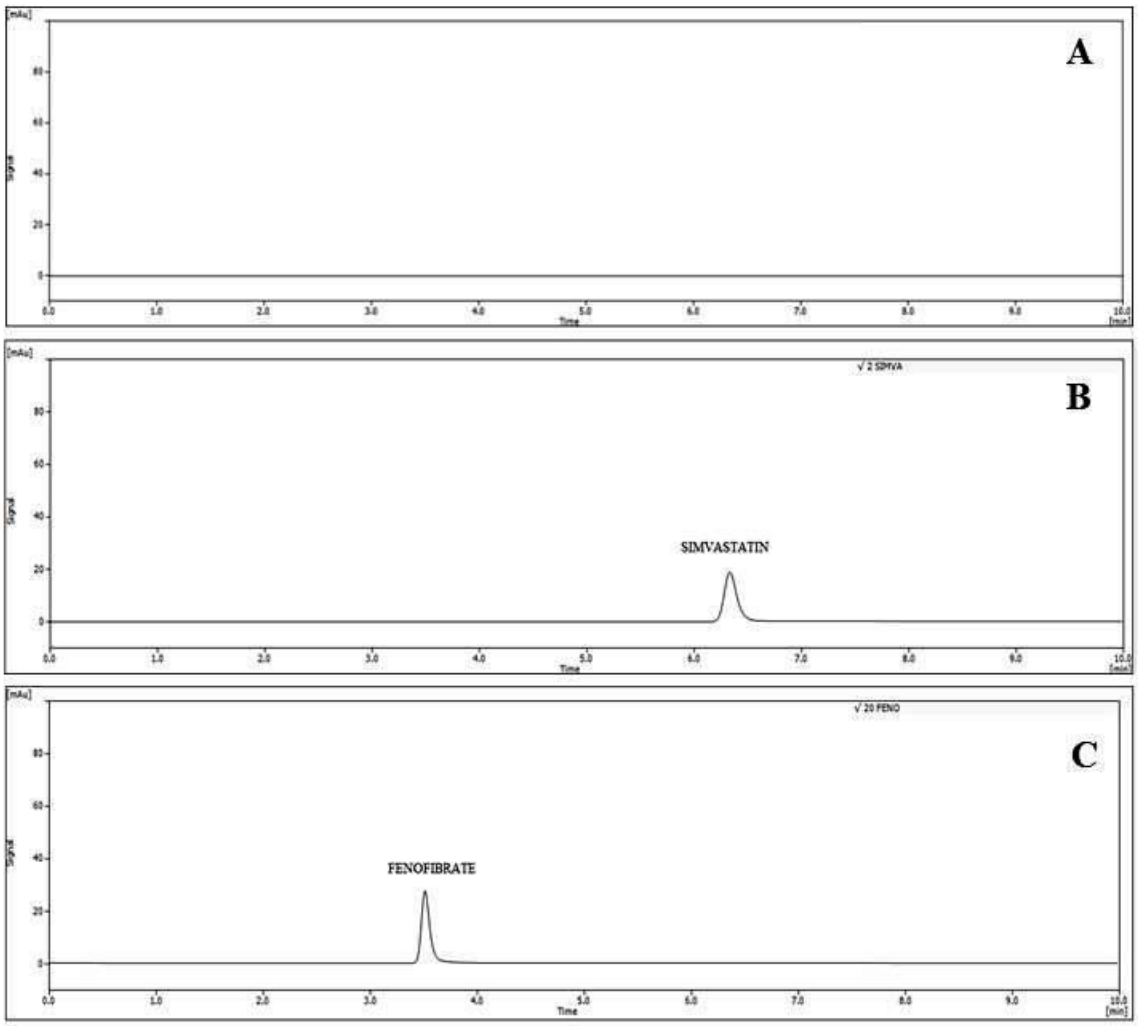

Figure 5: RP-HPLC Chromatogram for (a) Blank, (b) Fenofibrate (20 µg/ml) and (c) Simvastatin (2 µg/ml) in Acetonitrile: Water (pH 3.3): (90:10 % v/v) at 230 nm {Run time: 10 min, Flow rate: 1ml/min}

Linearity and Range

The Calibration curve was constructed with concentrations 1-5 µg/ml of Simvastatin and 10-50 µg/ml of Fenofibrate (n=6) for RP-HPLC (Figure 6) and UV methods (Figure 7 and 8).

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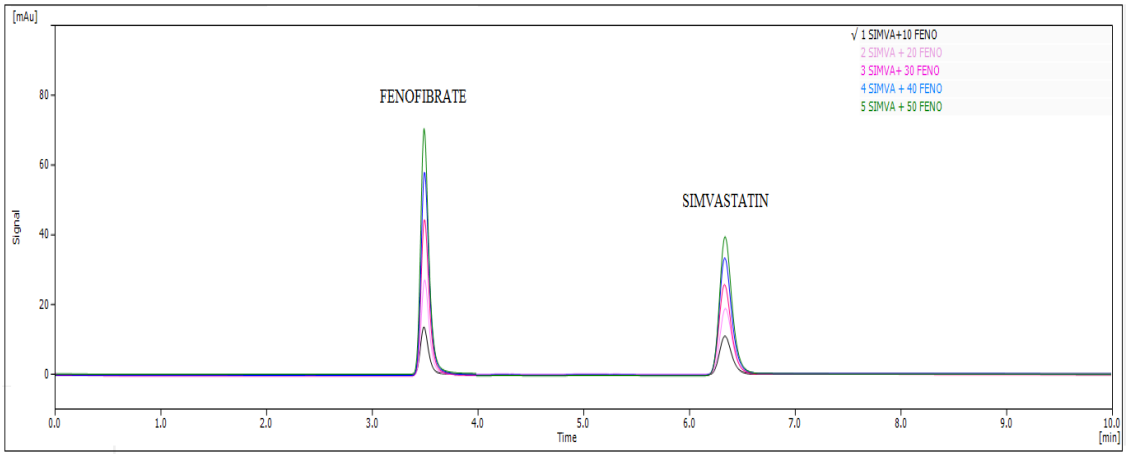

Figure 6: Overlain RP-HPLC Chromatogram of Fenofibrate (10-50 µg/ml) and Simvastatin (1-5 µg/ml) in Acetonitrile: Water (pH 3.3): (90:10 % v/v) at 230 nm {Run time: 10 min, Flow rate: 1 ml/min}

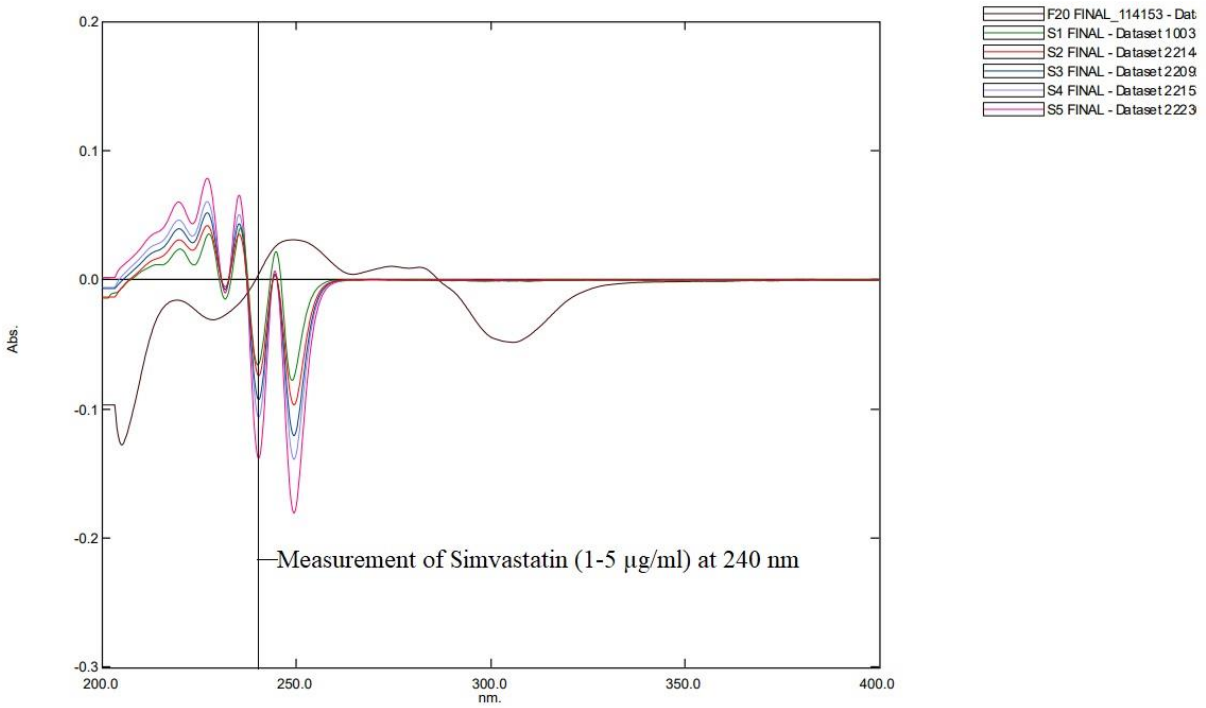

Figure 7: Overlain UV Spectra of Simvastatin (1-5 µg/ml) (Linearity) at 240 nm

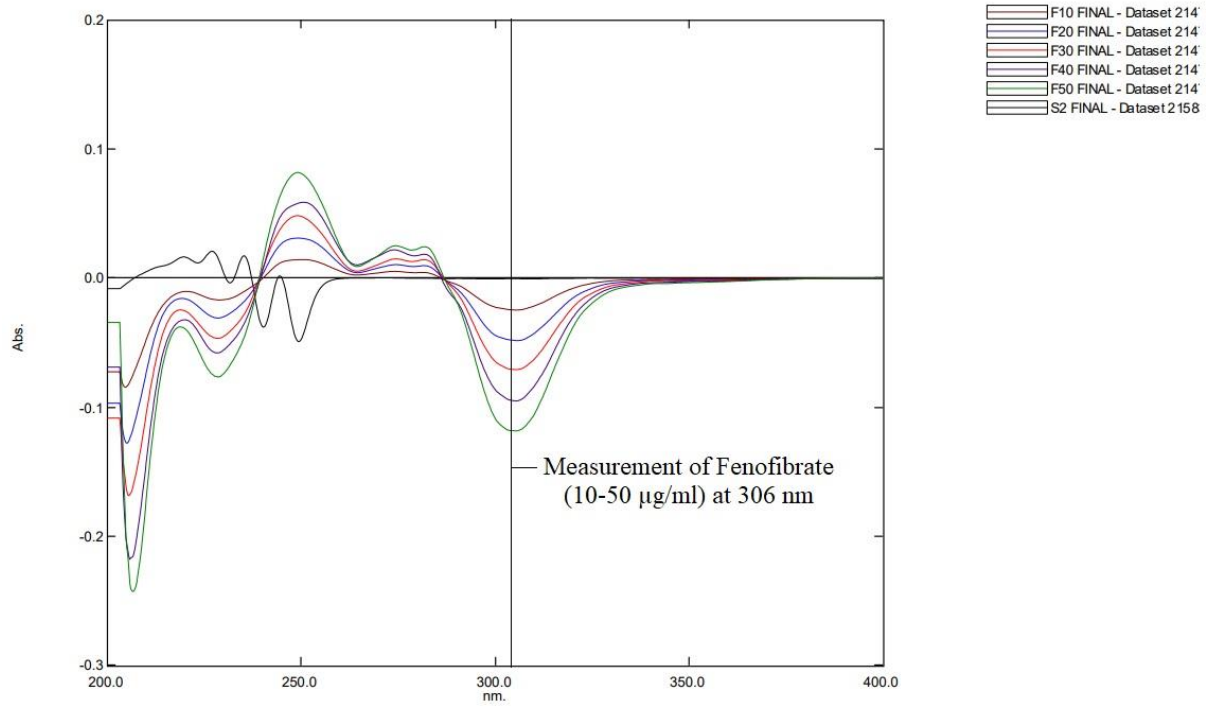

Figure 8: Overlain UV Spectra of Fenofibrate (10-50 µg/ml) (Linearity) at 306 nm

Aliquots of stock solution of Simvastatin (100 µg/ml) i.e., 0.1, 0.2, 0.3, 0.4, and 0.5 ml and for Fenofibrate (100 µg/ml) i.e., 1, 2, 3, 4 and 5 ml were pipetted in 10 ml of volumetric flask. Further diluted with diluent to obtain the different concentration like 1, 2, 3, 4 and 5 µg/ml for Simvastatin and 10, 20, 30, 40 and 50 µg/ml for Fenofibrate. Linearity was evaluated by linear regression analysis in terms of slope, intercept, and correlation coefficient.

Accuracy

Recovery study of RP-HPLC and UV method were conducted as per ICH guideline to determine accuracy at three different concentration levels i.e., 50 %, 100 % and 150 %. Solutions containing 2 µg/ml of

Simvastatin and 20 µg/ml of Fenofibrate were analyzed as 100 %. This performance was done in triplicate. Accuracy was calculated in percentage of recovery by standard addition method.

Precision

The precision studies of RP-HPLC and UV method were conducted at three levels like Intermediate (Intraday) precision, Reproducibility (Interday precision) and Repeatability. In Intraday precision, solutions containing 1, 2, 3 µg/ml of Simvastatin and 10, 20, 30 µg/ml of Fenofibrate were analyzed three times on the same day. In Interday precision, solutions containing 1, 2, 3 µg/ml of Simvastatin and 10, 20, 30 µg/ml of Fenofibrate were analyzed on three different successive days and in Repeatability, solutions containing 2 µg/ml of Simvastatin and 20 µg/ml of Fenofibrate were analyzed for six times. All the results were expressed in % R.S.D.

Detection Limit (DL) and Quantification Limit (QL)

Detection limit and Quantification limit of RP-HPLC and UV method were calculated using following equation as per ICH guidelines.

$$\text{Detection limit} = 3.3 \times \left(\frac{\sigma}{S}\right)$$

$$\text{Quantification limit} = 10 \times \left(\frac{\sigma}{S}\right)$$

Where, σ = standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

Robustness

The Robustness of the RP-HPLC method was determined by analysis of samples under a variety of conditions as flow rate (± 0.2 ml/min), wavelength (± 2 nm), and mobile phase ratio (± 2 % v/v).

System suitability tests

A system suitability test (Resolution, Column efficiency, tailing factor and Theoretical plates) were performed to verify resolution and reproducibility of chromatography system.

Forced degradation studies

Selectivity was assessed by performing Forced degradation studies. Combination of Simvastatin (2 µg/ml) and Fenofibrate (20 µg/ml) used as sample was stressed under various conditions like acid, alkaline, oxidative, photo and thermal to conduct forced degradation studies. Although, Simvastatin and Fenofibrate are practically soluble in Acetonitrile: Water (pH 3.3) (90:10; % v/v) was used as a solvent throughout studies.

Acid degradation

Accurately 1 ml of sample solution pipetted out from mixture solution in to 10 ml volumetric flask. 1 ml of 0.1 N Hydrochloric acid added to each flask and kept for 2 h at 40 ˚C. To neutralize, 1 ml of 0.1 N Sodium hydroxide was added in each flask and dilute upto volume with methanol. Filter the solution through 0.45-micron membrane filter and injected into chromatography and chromatogram has been recorded.

Base degradation

Accurately 1 ml of sample solution pipetted out from mixture solution in to 10 ml volumetric flask. 1 ml of 0.1 N Sodium hydroxide added to each flask and kept for 2 h at 40 ˚C. To neutralize, 1 ml of 0.1 N Hydrochloric acid was added in each flask and dilute upto volume with methanol. Filter the solution through 0.45-micron membrane filter and injected into chromatography and chromatogram has been recorded.

Oxidative degradation

Accurately 1 ml of sample solution pipetted out from mixture solution in to 10 ml volumetric flask. 1 ml of 3 % Hydrogen peroxide added to each flask and kept for 2 h at 40 ˚C. Filter the solution through 0.45-micron membrane filters and injected into chromatography and chromatogram has been recorded.

Photolytic degradation

Drugs were placed in a photo stability chamber and exposed to direct UV light for 2 h. At different time intervals the drugs were taken out, dilute appropriately and injected into chromatography to determine the amount of degradation of the drugs.

Thermal degradation

Accurately 1 ml of sample solution pipetted out from mixture solution in to 10 ml volumetric flask and exposed under heat at 80 ºC for 2 h. At different time intervals, make volume up to the mark with methanol and injected into chromatography to determine the amount of degradation of the drugs.

Statistical comparison of RP-HPLC and UV Method

The Student's t-test calculated using following formula:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{(S^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right))}}$$

where, t is the t-value, x_1 and x_2 are the means of HPLC and UV respectively, s^2 is the pooled standard error of the two groups, and n_1 and n_2 are the number of observations in each of the groups.

RESULTS AND DISCUSSION

RP-HPLC method

In order to select mobile phase, various solvents with different proportions as Acetonitrile: Water, Methanol: Water, Acetonitrile: Phosphate buffer were used. Resulting, Acetonitrile: Water (pH 3.3) (90:10; % v/v) has been selected as optimized mobile phase based on peak parameters which obeyed ideal system suitability parameters like proper migration, separation and resolution at flow rate (1 ml/min) at 230 nm of Simvastatin and Fenofibrate (Figure 9).

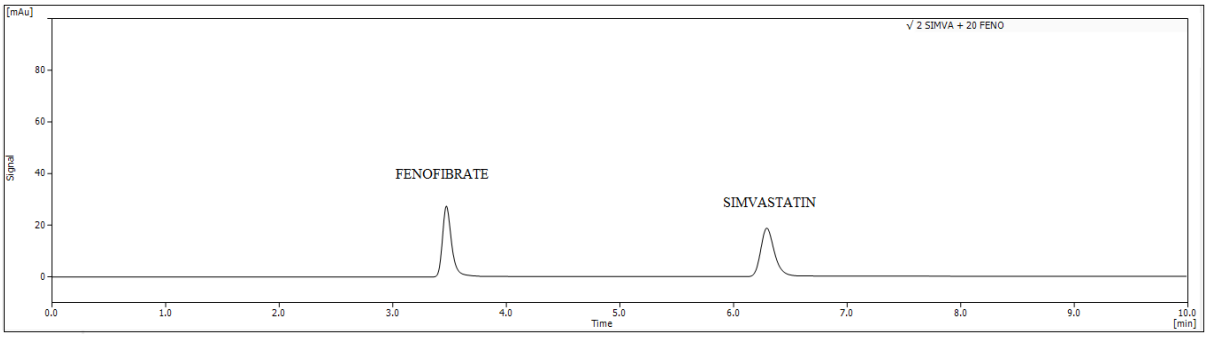

Figure 9: Optimized RP-HPLC Chromatogram of Fenofibrate (20 µg/ml) and Simvastatin (2 µg/ml) in ACN: Water (pH 3.3) (90:10 % v/v); Flow rate: 1 ml/min at 230 nm

Figure 9 showed, Fenofibrate and Simvastatin were eluted and forming symmetrical peaks, also well separated from solvent front. The Retention time of Fenofibrate and Simvastatin were observed at 3.5 and 6.3 min, allows a rapid determination of the drugs, which was important for routine analysis. The results of system suitability parameters were tabulated in table 1.

Table 1: System suitability parameter

Parameters	Retention Time	Tailing Factor	Number of Theoretical plates	Resolution
Fenofibrate	3.5	1.2	5584	
Simvastatin	6.3	1.6	6613	2.5

Calibration curve were constructed by plotting average Peak area versus Concentration. Straight line equations were obtained from calibration curve. The linear regression equation for Simvastatin was y = 100425x + 58995, with correlation coefficient (r= 0.9995), and y = 7113.1x + 33816, with correlation coefficient (r= 0.9993) for Fenofibrate which showed highly significant for the method (Table 2).

Table 2: Regression analysis of data for the quantitation of Simvastatin and Fenofibrate by the proposed methods

Statistical parameters	HPLC Method		UV Method	
Parameters	Simvastatin	Fenofibrate	Simvastatin	Fenofibrate
Concentration range (µg/ml)	1-5	10-50	1-5	10-50
Wavelength (nm)	230 nm		240 nm	306 nm

Regression equation (y = mx +c)	y = 100425x + 58995	y = 7113.1x + 33816	y = -0.0466x + 0.0116	y = -0.0047x + 0.0032
Correlation coefficient (r)	0.9995	0.9993	0.9991	0.9998

The % recovery of Simvastatin and Fenofibrate was found to be 99.50-100.02% and 99.86-100.05%, respectively (Table 3).

Table 3: Recovery test for Simvastatin and Fenofibrate

	% Level Of Recovery	Test Amount (µg/ml)	Amount of drug taken (µg/ml)	Total Spiked Std Amount (µg/ml)	Total amount Recovered (µg/ml)	% Recovery ±S.D. (n=3)	Total amount Recovered (µg/ml)	% Recovery ±S.D. (n=3)
					HPLC Method		UV Method	
Simvastatin	50	2	1	3	2.99	99.66±1.0561	2.98	99.33±1.014
	100	2	2	4	3.98	99.50 ±1.0715	3.95	98.75±1.193
	150	2	3	5	5.01	100.02±1.0902	4.99	99.80±1.142
Fenofibrate	50	20	10	30	29.96	99.86±1.1015	29.83	99.43±0.831
	100	20	20	40	40.02	100.05±1.0985	39.66	99.15±1.232
	150	20	30	50	50.02	100.04±0.8796	49.83	99.66±1.142

From the results, good sensitivity has been achieved which reflects the high efficiency of the separation methods. The intraday, interday and repeatability precision of Simvastatin and Fenofibrate were expressed in % RSD and indicated in acceptable limits. This result indicates that the method is precise and accurate. The precision data of Simvastatin and Fenofibrate showed in table 4 and table 5, respectively.

Table 4: Precision for Simvastatin

Intraday Precision of Simvastatin				
	HPLC Method		UV Method	
Conc. (µg/ml)	Mean Peak area ± SD (n=3)	% R.S.D.	Mean Absorbance ± SD (n=3)	% R.S.D.
	230 nm		240 nm	
1	155261.2±605.51	0.39	|-0.060| ± 0.0006	1.16
2	262558.1±945.20	0.36	|-0.103| ± 0.0009	0.87
3	364292.5±947.16	0.26	|-0.152| ± 0.0012	0.79
Interday Precision of Simvastatin				
Conc. (µg/ml)	Mean Peak area ± SD (n=3)	% R.S.D.	Mean Absorbance ± SD (n=3)	% R.S.D.
	230 nm		240 nm	
1	155360.3±621.44	0.40	|-0.062|± 0.0011	1.77
2	262595.2±919.08	0.35	|-0.105| ± 0.0012	1.14
3	364347.2±947.30	0.26	|-0.160| ± 0.0017	1.06
Repeatability of Simvastatin				
Conc. (µg/ml)	Mean Peak area ± SD (n=6)	% R.S.D.	Mean Absorbance ± SD (n=6)	% R.S.D.
	230 nm		240 nm	
2	262684.5±998.20	0.38	|-0.101| ±0.0008	0.79

Table 5: Precision for Fenofibrate.

Intraday Precision of Fenofibrate				
	HPLC Method		UV Method	
Conc. (µg/ml)	Mean Peak area ± SD (n=3)	% R.S.D.	Mean Absorbance ± SD (n=3)	% R.S.D.
	230 nm		306 nm	
10	107236.2±343.15	0.32	|-0.054| ± 0.0009	1.66
20	172538.5±414.09	0.24	|-0.096| ± 0.0011	1.15
30	249180.3±473.44	0.19	|-0.139| ± 0.0012	0.86
Interday Precision of Fenofibrate				
Conc. (µg/ml)	Mean Peak area ± SD (n=3)	% R.S.D.	Mean Absorbance ± SD (n=3)	% R.S.D.
	230 nm		306 nm	
10	107298.7 ± 354.08	0.33	|-0.048| ± 0.0009	1.88
20	172565.2 ± 448.66	0.27	|-0.099| ± 0.0011	1.11
30	249208.3 ± 498.41	0.20	|-0.148| ± 0.0013	0.88
Repeatability of Fenofibrate				
Conc. (µg/ml)	Mean Peak area ± SD (n=6)	% R.S.D.	Mean Absorbance ± SD (n=6)	% R.S.D.
	230 nm		306 nm	
20	172625.3 ± 414.30	0.24	|-0.098| ±0.0008	0.90

The Detection and Quantitation limit of Simvastatin were found to be 0.0231 µg/ml and 0.07 µg/ml, respectively and for Fenofibrate, Detection and Quantitation limit were found to be 0.2937 µg/ml and 0.89 µg/ml, respectively at 230 nm which were within the acceptable limits. The % assay of Simvastatin and Fenofibrate were found to be 100.08% and 99.99%, respectively. The Robustness was determined under a variety of conditions as flow rate (± 0.2 ml/min), wavelength (± 2 nm), and mobile phase ratio (± 2 % v/v) and results were expressed in % RSD. The Robustness data showed in table 6.

Table 6: Robustness Study for Simvastatin and Fenofibrate

Sr. No.	Parameter	Variation	% Assay ± S.D. (n=3)	
			Simvastatin	Fenofibrate
1	Flow rate (1 ml/min) (± 0.2 ml/min)	0.8 ml/min	99.01±604.51	97.75±702.37
		1.0 ml/min	99.50±699.42	97.65±750.55
		1.2 ml/min	99.85±706.47	97.25±781.02
2	Detection wavelength (230 nm) (± 2 nm)	228 nm	97.58±680.12	97.25±709.45
		230 nm	100.12±699.42	100.25±750.55
		232 nm	98.75±702.04	98.75±763.76
3	Mobile phase (90:10 %v/v) (± 2 %v/v)	88 :12 %v/v	98.12±652.07	97.75±721.11
		90 :10 %v/v	99.75±699.42	99.75±750.55
		92 :08 %v/v	99.95±720.14	98.25±757.18

UV Method

A reliable, precise and accurate UV spectrophotometric method was developed and validated for simultaneous estimation of Simvastatin and Fenofibrate in combination. Simvastatin (2 µg/ml) and Fenofibrate (20 µg/ml) solutions were scanned between 200-400 nm.

The detection wavelength (λ) for Simvastatin and Fenofibrate were found to be 240 nm and 306 nm, respectively. These wavelengths were used for all measurements. The UV spectra of Simvastatin (1-5 µg/ml) and Fenofibrate (10-50 µg/ml) were constructed and the linearity range were observed (Figure 7 and 8). Calibration curves were constructed and Beer's law was obeyed over the concentration range of 1-5 µg/ml for Simvastatin and 10-50 µg/ml for Fenofibrate. The linear regression equation (correlation coefficient) for Simvastatin were y = -0.0466x + 0.0116 at 240 nm (r = 0.9991) and for Fenofibrate y = -0.0047x + 0.0032 at 306 nm (r = 0.9998) (Table 2). The % recovery of Simvastatin and Fenofibrate was found to be 99.80 - 99.33% and 99.66 - 99.15%, respectively (Table 3). Results were obtained lie in acceptable limits. From the results, good sensitivity has been achieved which reflects the high efficiency of the separation methods. The intraday, interday and repeatability precision of Simvastatin and Fenofibrate were expressed in % RSD and indicated in acceptable limits. This result indicates that the method is precise and accurate. The precision study of Simvastatin and Fenofibrate showed in table 4 and table 5, respectively.

The Detection and Quantitation limit of Simvastatin were found to be 0.08 µg/ml and 0.24 µg/ml at 240 nm and for Fenofibrate, Detection and Quantitation limit were found to be 0.64 µg/ml and 1.92 µg/ml at 306 nm which were within the acceptable limits. The % assay of Simvastatin and Fenofibrate were found to be 99.50% and 99.75 %, respectively.

FORCED DEGRADATION STUDIES

Peak area of Fenofibrate and Simvastatin were found to be 172545.5 and 262560.3, respectively. % degradation of Fenofibrate and Simvastatin were calculated using this equation,

$$\%\ degradation = 100 - \left(\frac{Degradation\ area}{Standard\ area}\right) \times 100$$

Acid degradation study

The combination showed sufficient degradation within 2 h with 0.1 N Hydrochloric acid at 40˚C. Fenofibrate showed 13.26 and 16.77 % degradation at 1 and 2 h, respectively; whereas Simvastatin showed 8.76 and 14.47 % degradation at 1 and 2 h, respectively (Figure 10).

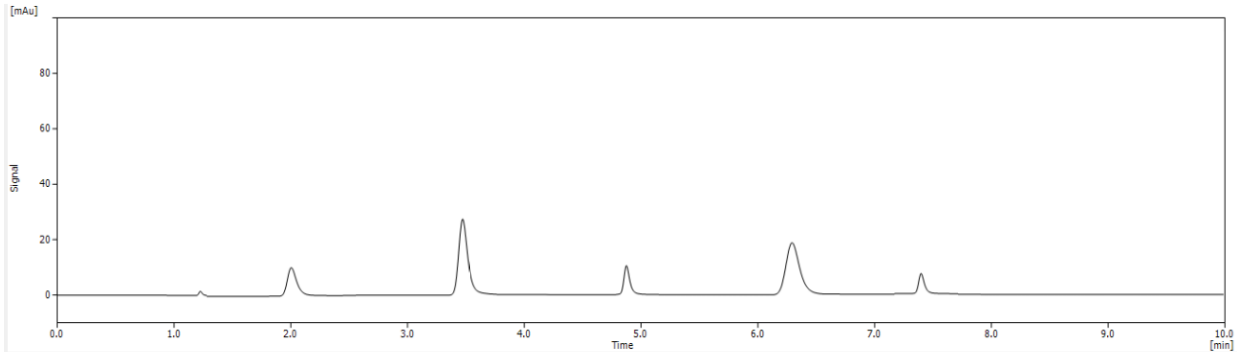

Figure 10: RP-HPLC Chromatogram of Acid Degradation for Fenofibrate (20 µg/ml) and Simvastatin (2 µg/ml) Sample at 2 h at 230 nm {Run time: 10 min, Flow rate: 1ml/min}

Base degradation study

Similar to acid, sufficient degradation was observed within 2 h with 0.1 N Sodium Hydroxide at 40˚C. Fenofibrate showed 16.98 and 18.93 % degradation at 1 and 2 h, respectively; whereas Simvastatin showed 15.72 and 18.46 % degradation at 1 and 2 h, respectively (Figure 11).

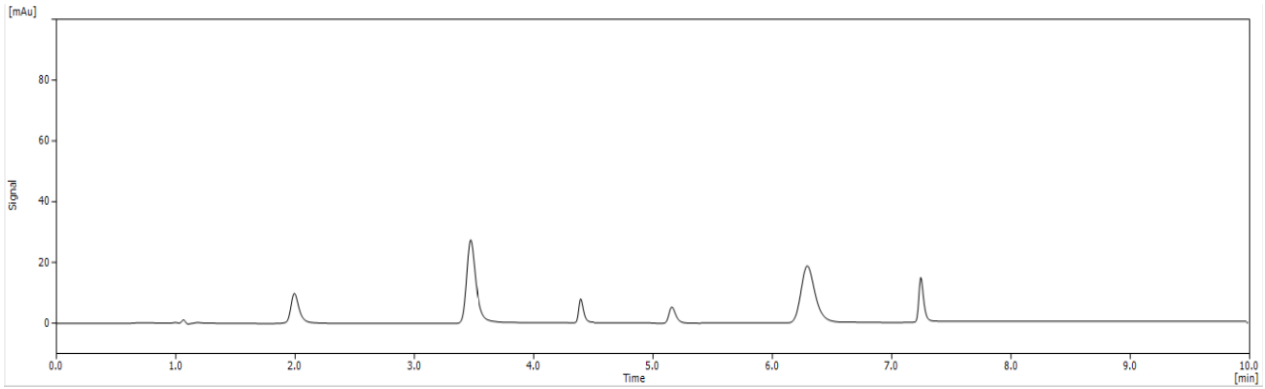

Figure 11: RP-HPLC Chromatogram of Base Degradation for Fenofibrate (20 µg/ml) and Simvastatin (2 µg/ml) Sample at 2 h at 230 nm {Run time: 10 min, Flow rate: 1ml/min}

Oxidative degradation study

Degradation was observed within 2 h after heating with 3 % Hydrogen peroxide at room temperature. Fenofibrate showed 7.45 and 15.55 % degradation at 1 and 2 h, respectively; whereas Simvastatin showed 6.08 and 12.44 % degradation at 1 and 2 h, respectively (Figure 12).

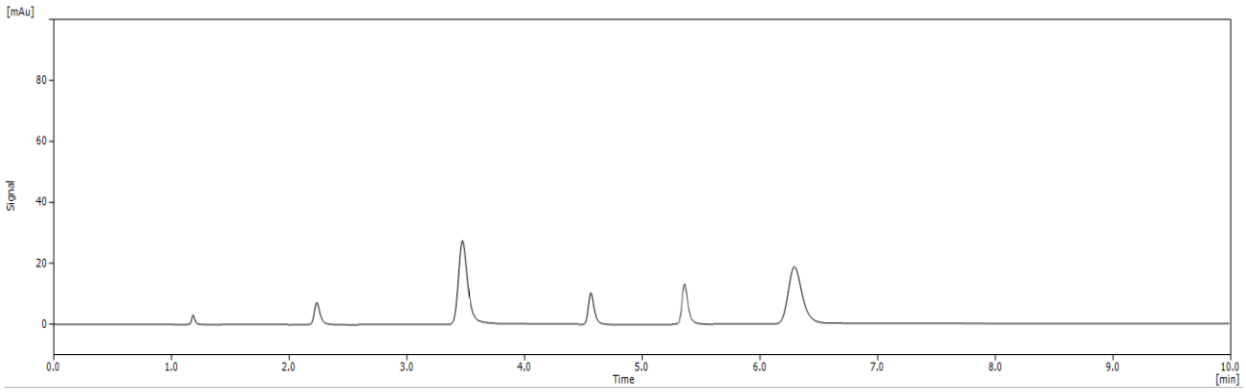

Figure 12: RP-HPLC Chromatogram of Oxidative Degradation for (20 µg/ml) and Simvastatin (2 µg/ml) Sample at 2 h at 230 nm {Run time: 10 min, Flow rate: 1ml/min}

Photolytic degradation study

Drugs were exposed to direct UV light for 2 h. Fenofibrate showed 6.00 and 13.58 % degradation at 1 and 2 h, respectively; whereas Simvastatin showed 4.38 and 8.64 % degradation at 1 and 2 h, respectively (Figure 13).

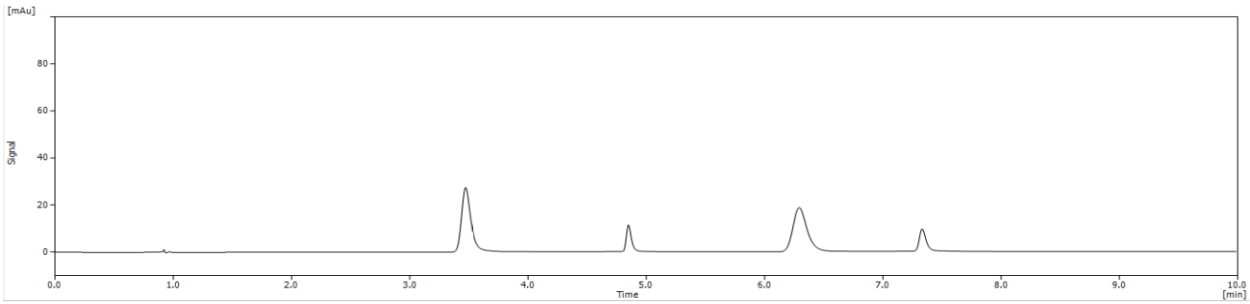

Figure 13: RP-HPLC Chromatogram of Photolytic Degradation for (20 µg/ml) and Simvastatin (2 µg/ml) Sample at 2 h at 230 nm {Run time: 10 min, Flow rate: 1 ml/min}

Thermal degradation study

Drugs were exposed under heat at 80 ºC for 2 h. Fenofibrate showed 5.80 and 11.38 % degradation at 1 and 2 h, respectively; whereas Simvastatin showed 3.81 and 7.63 % degradation at 1 and 2 h, respectively (Figure 14).

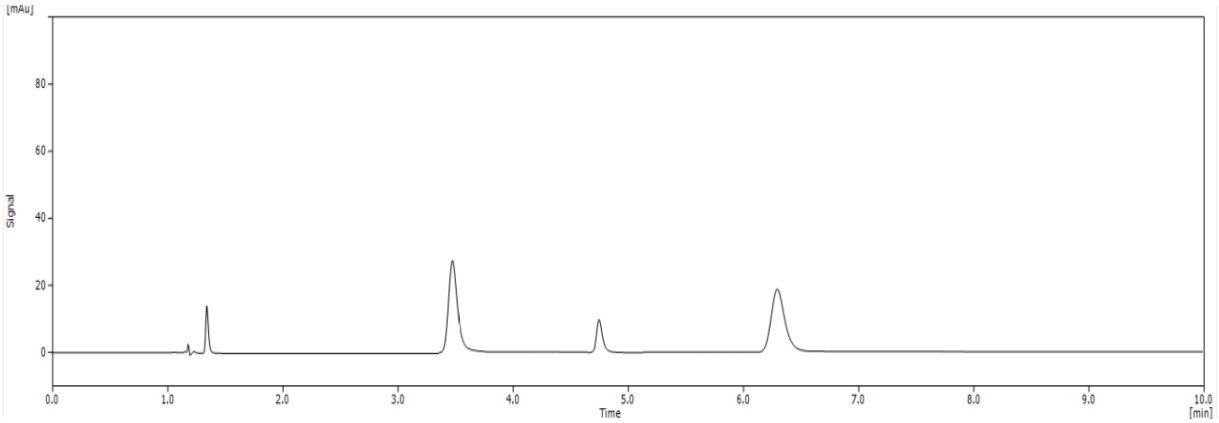

Figure 14: RP-HPLC Chromatogram of Thermal Degradation for Fenofibrate (20 µg/ml) and Simvastatin (2 µg/ml) Sample at 2 h at 230 nm {Run time: 10 min, Flow rate: 1 ml/min}.

Statistical comparison of RP-HPLC and UV Method

The proposed analytical methods were compared using Statistical analysis. The student's t-test was applied and did not show significant difference between experimental values obtained in sample analysis by the two methods. The calculated t-value ($t_{calculated}$) was smaller than critical t-value ($t_{tabulated}$ / $t_{critical}$), at 5 % significance level.

CONCLUSION

Simple, rapid, sensitive, accurate and precise RP-HPLC and UV spectroscopic methods has been developed and validated for routine analysis of Simvastatin and Fenofibrate. These proposed methods were suitable for simultaneous estimation of Simvastatin and Fenofibrate in bulk drug and synthetic mixture without any interference. The developed and validated methods were successfully applied in combination. Comprehensive stress testing to mixture of Simvastatin and Fenofibrate was carried out according to ICH guideline Q1A (R2) under various stress conditions in the presence of degradation products. During degradation study, the results obtained were found within the acceptance criteria. Validation of proposed methods was also carried out according to ICH guideline Q2 (R2)[37-38]. Hence, the proposed stability indicating RP-HPLC assay method and UV method might be applied and utilized for the routine analysis for the estimation of Simvastatin and Fenofibrate in combination. Statistical analysis proved that the proposed methods were repeatable and selective for the analysis of Simvastatin and Fenofibrate in combination.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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