Original Article

Simultaneous estimation of simvastatin and fenofibrate from their combined dosage form by ultraviolet–visible spectroscopy using simultaneous equation method

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ABSTRACT

Objective: This research paper describes a simple, precise, and accurate UV-Vis Spectrophotometric method was developed and validated for simultaneous estimation of simvastatin (SIM) and fenofibrate (FEN) from their combination dosage form. **Method:** This method includes the formation and solving of a simultaneous equation using 238 nm and 287 nm as two analytical wavelengths (λ_{max} of the drugs) of detection. Both the drugs followed Beer-Lambert's law over the concentration range 0.60-3.60 µg/mL for simvastatin and 4.35-26.10 µg/mL for fenofibrate, respectively. **Results:** Validation of the new developed method was done by linearity, precision, accuracy, limit of detection, limit of quantitation and robustness as per ICH guidelines and the results of the analysis were validated statistically. **Conclusion:** The available information from the research will be very informative towards the multi-component analysis of these drugs and will open new paradigms in the upcoming research in the field of analysis.

Keywords: Fenofibrate, simultaneous equation method, simvastatin, ultraviolet–visible spectroscopy

INTRODUCTION

Fenofibrate (FEN) [Figure 1], a drug belongs to fibrate class, is 2-(4-[4-chlorobenzoyl] phenoxy)-2-methyl-propanoic acid, 1 methyl ethyl ester. It is used to the lower cholesterol levels in those who are at risk of cardiovascular disease. It decrease both low-density lipoprotein and very-low-density lipoprotein levels, as well as elevating high-density lipoprotein levels and lowering triglyceride levels, just like other fibrates.[1] It stimulates the peroxisome proliferator-activated receptor to become active. By activating lipoprotein lipase and decreasing apoprotein C-III synthesis, this enhances lipolysis and eliminates triglyceride-rich particles from plasma.[2] FEN is a prodrug that is hydrolyzed into its active main metabolite through tissue and plasma esterases shortly after absorption.[3,4] Simvastatin (SIM) [Figure 2] is a lipid-lowering medication. The enzyme 3-hydroxy-3-methylglutarylcoenzyme (HMG-CoA) reductase is inhibited by it. The conversion of HMG-CoA to mevalonate, an early and rate-limiting step in the production of cholesterol, is catalyzed by this enzyme. SIM is a prodrug that, after absorption, undergoes fast hydrolysis to produce a series of active metabolites in humans. When it comes to inhibiting HMG-CoA reductase, the main metabolite, β-hydroxy acid-SIM, is the most effective. SIM is absorbed into the human body and undergoes extensive first-pass metabolism in the liver, the principal site of action, before being excreted in the bile. As a result, the drug's systemic bioavailability is low: β-hydroxyacid-SIM has an absolute bioavailability of <5%.[5-7] A thorough review of the literature indicated that several analytical procedures like spectroscopic method.[8-15] High-performance liquid chromatography (HPLC) method,[16-19] high-performance thin-layer chromatography,[20,21] ultra performance liquid chromatography[22,23] are available for the determination of these drugs individually or in combination with other drugs. At this time, no ultraviolet (UV) spectrophotometric or HPLC methods for simultaneous measurement of FEN and SIM in bulk or tablet dosage form have been reported. As a result, a simple, precise, and accurate UV-spectrophotometric and reversed-phase HPLC method for the simultaneous measurement of FEN and SIM in bulk and tablet dosage form was developed. The developed method was

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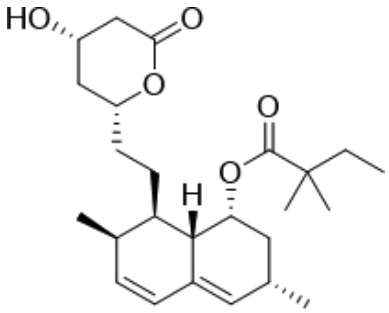

Figure 1: Chemical structure of simvastatin

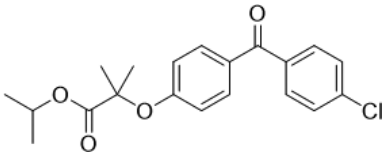

Figure 2: Chemical structure of fenofibrate

validated in accordance with International Council on Harmonisation (ICH) recommendations.[24] The development and validation of a UV-Spectrophotometric and HPLC method for simultaneous measurement of FEN and SIM in a bulk and tablet formulation are described in this paper.

MATERIALS AND METHODS

Chemicals and reagents

SIM API was purchased from an online chemical selling website, that is, Carbanio.com, whereas FEN was obtained as a gift sample from LA Pharmachem Private Limited, Ludhiana, Punjab, India. Tablets containing SIM (20 mg) and FEN (145 mg) were prepared in the Department of Pharmaceutics, ISF College of Pharmacy, Moga, Punjab. All chemicals used were AR grade.

Instrumentation

A double beam ultraviolet–visible (UV-Vis) spectrophotometer (Shimadzu model UV 1700 PC, Shimadzu Corporation, Tokyo, Japan) with a spectral width of 2 nm, quartz cell (1.0 cm path) was employed to measure the absorbance of solutions. Based on the solubility, study methanol was selected as the solvent for dissolving SIM and FEN.

Standard stock solutions of SIM and FEN

Standard stock solution of drugs was prepared individually containing 1000 µg/mL of each drug. The solutions were filtered through 0.45 µm Whatman filter paper.

Determination of λ_{max} of individual components

By appropriate dilution of standard solutions of SIM and FEN with methanol, solutions containing 1.2 µg/mL for SIM and 8.7 µg/mL for FEN were scanned separately in the range of 200–400 nm against methanol as blank. SIM shows λ_{max} at 238 nm and FEN shows λ_{max} at 287 nm [Figure 2].

Overlay spectra of SIM and FEN

The overlain spectra of SIM and FEN were recorded [Figure 3] and two wavelengths 238 nm (λmax of SIM) and 287 nm (λmax of FEN) were selected for subsequent study.

Methods: Simultaneous equation method

In methanol, standard stock solutions of SIM and FEN in the concentration ranges of 0.60–3.60 g/mL and 4.35–26.10 g/mL were prepared, and their absorbance was measured at 238–287 nm, respectively. Calibration curves were plotted to confirm Beer's law. Using these absorptivity data, two simultaneous equations were developed. A (1%, 1 cm).[25]

From Beer-Lambert Law

A= abc

At λ_1

$$A_1 = ax_1bc_x + ay_1bc_y \quad (1)$$

At λ_2

$$A_2 = ax_2bc_x + ay_1bc_y \quad (2)$$

If Measurement in 1 cm, b=1 then rearrange equation (2)

$$C_y = \frac{A_2 - ax_2c_x}{ay_2}$$

Putting the value of Cy in eq. (1) and rearranging gives

$$C_x = \frac{(A_2ay_1 - A_1ay_2)}{(ax_2ay_1 - ax_1ay_2)}$$

and

$$C_y = \frac{(A_1ax_2 - A_2ax_1)}{(ax_2ay_1 - ax_1ay_2)}$$

Where Cx and Cy are the concentration of SIM and FEN, respectively, A1 and A2 are absorbance's at 238–287 nm, respectively, ax1 and ax2 are absorptivity of SIM at 238–287 nm, respectively; ay1 and ay2 are absorptivity of FEN at 238–287 nm, respectively. By solving the two simultaneous equations, the concentrations of SIM and FEN in sample solutions were obtained.

Calculation of absorptivity value

The absorptivity value of FEN and SIM from each solution was calculated using the formula below and the results are shown in Table 1.[10]

$$\text{Absorptivity} = \frac{\text{Absorbance}}{\text{Concertration}}(\text{g}/100\text{ml})$$

Analysis of tablet formulation

Average weight of 20 tablets was measured and then crushed to a fine powder. Average power equivalent to 2 mg of SIM (also contain 14.5 mg of FEN) was weighed accurately and was transferred to a 100 mL volumetric flask. To this 20 mL of methanol was added and shaken for 30 min and sonicated for 10 min. Final volume was added up to 100 mL with the same solvent. The solution was filtered through Whatman filter paper. 10 mL of the above solution was diluted to 100 ml with

methanol. They contained 2 μg/ml of SIM and 14.5 μg/ml of FEN. The absorbance of the solution was measured at 238–287 nm and the amount of SIM and FEN present in each tablet was found to be 19.85–146.2 mg, respectively. The assay results are described in Tables 1-7.

Finally, the newly developed simple simultaneous equation approach was successfully applied to the tablet dosage form, with the assay results demonstrating that this method utilized to estimate both medications in a combined dose form.

Validation of proposed method

The method has been validated for linearity, sensitivity, accuracy, and precision for each analyte using ICH guidelines for validation of analytical techniques.[26]

Linearity

In the concentration ranges of 0.60–3.60 μg/mL and 4.35–26.10μg/mL, respectively, appropriate dilutions of working standard solutions for SIM and FEN were prepared and evaluated according to the developed method. The linearity of the calibration curves was determined using the least square regression approach.

Precision

Intraday and interday variations were used to assess precision. SIM (0.60, 1.20, and 1.8 μg/mL) and FEN (4.35, 8.70, and 13.05 μg/mL) were tested 3 times on the same day to determine intraday precision. By analyzing the same concentration of solutions on 3 distinct days, interday precision was determined.

Accuracy (recovery studies)

A recovery study was carried out to investigate the accuracy by adding standard drug solutions at three concentration levels (80%, 100%, and 120%) to a pre-analyzed sample.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of the developed method were calculated to form the linearity results using this formula.

Table 1: Absorbance and Absorptivity of SIM and FEN at two wavelengths

Conc. of sol. (μg/mL)		Absorbance				Absorptivity			
		SIM		FEN		SIM		FEN	
SIM	FEN	238	287	238	287	238	287	238	287
0.60	4.35	0.166	0.011	0.049	0.127	0.276	0.018	0.011	0.029
1.20	8.70	0.298	0.023	0.089	0.218	0.248	0.019	0.010	0.025
1.80	13.05	0.439	0.035	0.129	0.332	0.243	0.019	0.009	0.025
2.40	17.40	0.578	0.05	0.172	0.436	0.240	0.020	0.009	0.025
3.0	21.75	0.702	0.061	0.207	0.523	0.234	0.020	0.009	0.024
3.60	26.10	0.831	0.074	0.254	0.632	0.230	0.020	0.009	0.024
Average						0.245	0.019	0.0095	0.0253

SIM: Simvastatin, FEN: Fenofibrate

The LOD may be calculated as

$$LOD = 3.3 \times SD/Slope$$

The LOQ may be calculated as

$$LOQ = 10 \times SD/Slope$$

Where, SD = Three replicates of absorbance

Slope = the mean slope of the three calibration curves

Robustness

Robustness of the method was studied by analyzing the tablet formulation at various conditions likes such as analyst to analyst variation and instrument to instrument variation.

RESULTS AND DISCUSSION

In the present study, we have to develop a UV-Vis spectrophotometric method for the simultaneous estimation of SIM and FEN from the combined dosage form. The developed method was validated as per the ICH guidelines.

Linearity

A linear relationship was found in the concentration range of 0.60–3.60 μg/mL for SIM [Figures 4 and 5] and 4.35–26.10 μg/mL for FEN at each wavelength, thst is, 237–287 nm [Figures 6 and 7]. The absorptivity was found approximately the same for all the concentrations hence both drugs obeyed Beer Lambert's law in indicated concentration range. The high value of the correlation coefficient (R2) also indicates good linearity for both drugs. The absorbance's were measured at the selected wavelengths and absorptivity for both drugs was determined at both wavelengths [Table 1]. Regression analysis results are given in Table 2.

Accuracy

Results of recovery studies [Figure 8] are shown in Table 3. Percentage recovery for SIM and FEN by this method was found in the range of 98.86–100.41% and 99.73–100.57%, respectively.

Precision

Interday and intraday precision results are depicted in Table 4 which were found to be within the limits, that is, % RSD <2%.

LOD and LOQ

The values of LOD and LOQ are given in Table 5.

Table 2: Regression results

Parameters	SIM		FEN	
Wavelength (nm)	238	287	238	287
Beer's Law limit (μg /mL)	0.60–3.60	0.60–3.60	4.35–26.10	4.35–26.10
Regression equation (*Y)	y=0.2289× +0.0186	y=0.0208× –0.0011	y=0.0095× +0.0042	y=0.0239× +0.0126
Slope (m)	0.2289	0.0208	0.0095	0.0239
Intercept (c)	0.0186	0.0011	0.0042	0.0126
Correlation coefficient (r2)	0.9985	0.9988	0.9988	0.9984

SIM: Simvastatin, FEN: Fenofibrate

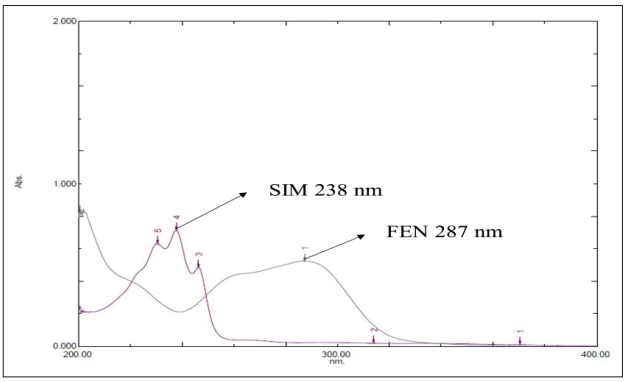

Figure 3: Overlain spectra of simvastatin and fenofibrate

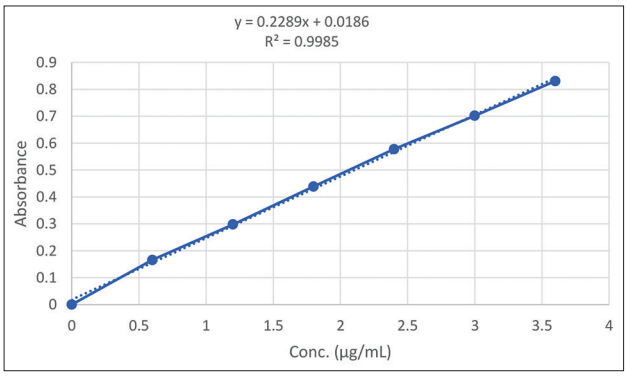

Figure 4: Calibration curve of the simvastatin at 238 nm

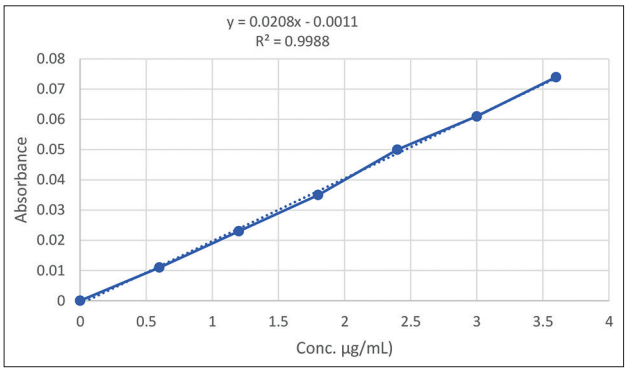

Figure 5: Calibration curve of the simvastatin at 287 nm

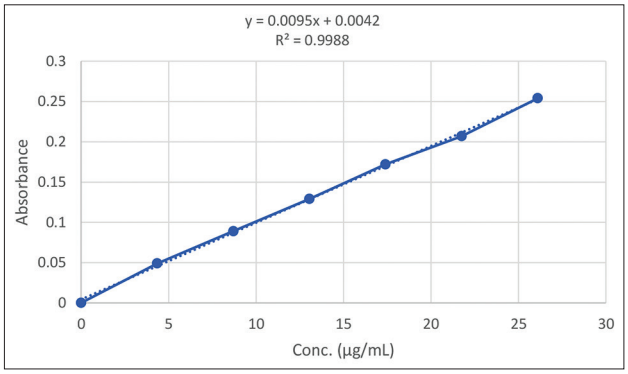

Figure 6: Calibration curve of fenofibrate at 238 nm

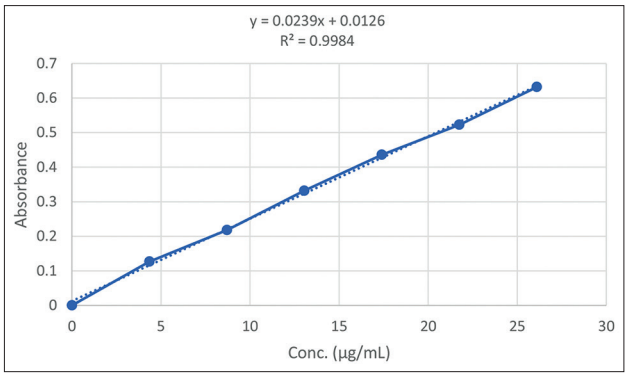

Figure 7: Calibration curve of fenofibrate at 287 nm

Table 3: Recovery study of SIM and FEN

Amount of sample (µg/mL)		Amount of drug added (µg/mL)		Percent of the spiked sample		Amount recovered (µg/mL)		Percent recovery	
SIM	FEN	SIM	FEN	SIM	FEN	SIM	FEN	SIM	FEN
1.20	8.70	0.96	6.96	80	80	2.14	15.63	99.07	99.80
1.20	8.70	1.20	8.70	100	100	2.41	17.50	100.41	100.57
1.20	8.70	1.44	10.44	120	120	2.61	19.09	98.86	99.73

SIM: Simvastatin, FEN: Fenofibrate

Table 4: Interday and intraday precision

Interday						Intraday					
Amount taken*		Amount found**		%RSD		Amount taken*		Amount found**		%RSD	
SIM	FEN	SIM	FEN	SIM	FEN	SIM	FEN	SIM	FEN	SIM	FEN
0.60	4.35	0.586	4.36	0.36	0.72	0.60	4.35	0.595	4.354	0.362	0.625
1.20	8.70	1.19	8.684	0.48	0.65	1.20	8.70	1.184	8.740	0.523	0.287
1.80	13.05	1.812	13.036	0.25	0.47	1.80	13.05	1.812	13.04	0.425	0.591

SIM: Simvastatin, FEN: Fenofibrate

Table 5: LOD and LOQ

Drugs	Wavelength	LOD (µg/ml)	LOQ (µg/ml)
SIM	238 nm	0.18	0.56
FEN	287 nm	1.19	3.61

LOD: Limit of detection, LOQ: Limit of quantification, SIM: Simvastatin, FEN: Fenofibrate

Table 6: Results of robustness study

Factor	Term	SIM		FEN	
		% Drug estimated	%RSD	% Drug estimated	% RSD
Analyst to analyst variation	Anal. 1	99.46	0.84	100.24	0.44
	Anal. 2	99.12	0.68	99.32	0.75
Instrument to instrument variation	Instr.1	98.89	0.65	98.45	0.65
	Instr.2	99.15	0.76	99.25	0.85

Concentration of SIM (1.20 µg/ml) and FEN (8.70 µg/mL) (n=3), SIM: Simvastatin, FEN: Fenofibrate

Robustness

Robustness of the method was studied by analyzing the tablet formulation at various conditions such as analyst to analyst variation and instrument to instrument variation [Table 6].

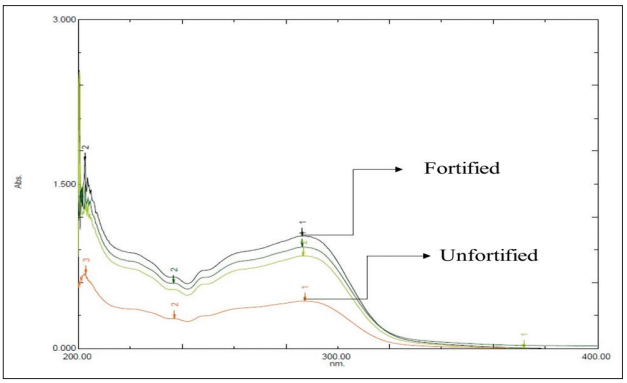

Figure 8: Overlay spectra of unfortified and fortified sample

Table 7: Assay results

Drug	Label claim (mg/Tab)	Amount Found (mg/Tab)	% Estimation (mg/Tab)
SIM	20	19.85	99.25
FEN	145	146.2	100.82

SIM: Simvastatin, FEN: Fenofibrate

Assay

The formulation (tablet) analysis revealed good agreement (99.25–100.82%) with label claim. Results of assay are depicted in Table 7.

CONCLUSION

The proposed UV-Vis spectrophotometric method is simple, specific, linear, accurate, precise, and robust. Hence, it can be used for the analysis of SIM and FEN in the combined dosage form.

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