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Simultaneous Equation, Absorbance Ratio and First Order Derivative Methods for the Estimation of Atorvastatin Calcium and Fenofibrate in Solid Dosage Form

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Abstract:- Three simple, precise, accurate, specific and reproducible spectrophotometric methods, requiring no prior separation, have been developed for the simultaneous estimation of atrovastatin calcium (ATV) and fenofibrate (FEN) in combined dosage form. Quantitative estimation of these drugs in marketed brands of the tablet was carried out using simultaneous equation, absorbance ratio and first order derivative methods. In the simultaneous equation method ATV and FEN have absorbance maxima at 247 and 289 nm respectively in methanol. Second method employs Q absorbance analysis using 254 nm (Isobestic point) and 289 nm (λ max of FEN) as two wavelengths for estimation. The Third method involves First order derivative spectroscopy using 236 nm and 246 nm as zero crossing points for ATV and FEN respectively. Linearity was observed in the concentration range of 0-24 µg/ml for both ATV and FEN. Accuracy and Precision of the method was determined and validated statistically. Method showed good reproducibility and recovery with % RSD less than 2%. The methods were successfully applied for determining the amount in marketed formulation.

Keywords:- *Atrovastatin Calcium, Fenofibrate, Simultaneous Equation, Absorbance Ratio, First Order Derivative.*

I. INTRODUCTION

Atrovastatin calcium (ATV) is a lipid lowering agents, chemically it is [R-(R*-R*)]-2-(4-fluorophenyl-β, γ-dihydroxy-5-(1-methyl ethyl)-3-phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate[1].Few HPLC[2,3,4,]CZE[5],GC-MS[6],LC-MS[7] and other methods[8] have been reported for its estimation. Fenofibrate (FEN) lipid lowering agent. It is official in BP[9]. Chemically it is 2-[(4-4-chlorobenzoyl) phenoxy] -2-methyl propionic acid, 1-methyl ethyl ester. Several UV derivative spectra, HPLC[10,] NMR [11], and spectrophotometric [12] methods have been reported for its estimation of FEN in body fluids and in dosage form.

Both these drugs are available in combined tablet dosage form as an antilipemic agent. The extensive literature survey revealed that numbers of methods are reported for the individual drugs and combination with other drugs but no method is so for reported for the simultaneous estimation of ATV and FEN in combined dosage form. So the attempts have been made to develop three simple, precise, accurate, specific and reproducible spectrophotometeric methods, for the simultaneous estimation of ATV and FEN in combined dosage form, using simultaneous equation, absorbance ratio and first order derivative methods.

II. MATERIALS AND METHODS

A. Reagent

Standard bulk drug sample of ATV and FEN were provided by M/S KREB'S Pharmaceuticals Chennai. A tablet of combined dosage form was procured from the local market (Atorlip-F). All other reagents used were of analytical grade. Shimadzu UV/ visible spectrophotometer, model 1700 and 1cm matched quartz cells was used. Spectra were recorded using program having following specifications spectral bandwidth 3 nm, wavelength accuracy +/- 0.5 nm, wavelength readability in 0.1 nm increments.

B. Experiment

- *Method 1: Employing Simultaneous Equations*

Pure drug sample of ATV and FEN were dissolved separately in methanol so as to give several dilutions of standard in the concentration range 0-24µg/ml of ATV and FEN. All dilutions were scanned in the wavelength range of 400-200nm. Fig-1 represents the overlain spectra of both the drugs.

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Two wavelength selected for the formation of simultaneous equations were 247 and 289nm (λ max of both the drugs respectively), E (1%, 1cm) determined for ATV at 247 and 289 nm were 447.34 and195.34 while respective values for FEN are309.13 and 583.32.These values are the mean of six independent determination. The simultaneous equations formed were,

- At λ_1 $A_1 = a\,x_1bc_x + ay_1bc_y$ ------ (1)
- A1 = 447.34 C_X + 309.13 C_Y------ (2)
- At λ_2 $A_2 = a\,x_2bc_x + ay_2bc_y$------- (3)
- A2 = 195.34 C_X +583.32 C_Y ------ (4)

Where A_1 and A_2 are the absorbances of sample solution at 247 and 289 nm respectively. Cx and C_Y are the concentration of ATV and FEN respectively (µg/ml) in sample solution.

The absorbance's ($A_{1&}$ A_2) of the sample solution were recorded at 247 and 289nm respectively and concentration of both the drugs were calculated using above mentioned equation (2&4).

➢ *Method II: Employing Absorbance Ratio (or) Q-Analysis Method***:**

From the above overlain spectrum of ATV and FEN, 2 wavelengths were selected one at 254 nm which was isoabsorptive point for both the drugs and other at 289 λ max of FEN. The absorbance of the standard and sample solutions were prepared and measured in the same manner as in the previous method. The absorptive values, the concentration of drugs in sample solution were determined by using the following formula.

- For ATV
- $C_1 = Q_0\text{-}Q_2 / Q1\text{-}Q \times A/a_1$

- For FEN
- $C_2 = Q_0\text{-}Q_1/Q2\text{-}Q_1 \times A/a_2$

- Q = absorbance of sample at 247nm /absorbance of sample at 254 nm

- Q_1 = absorptivity of ATV at 247nm / absorptivity of ATV at 254nm

- Q_2 = absorptivity of FEN at 247nm / absorptivity of FEN at 254nm

- A—absorbance of sample at isoabsorptive point, a_1&a_2 ---- absorptive of ATV and FEN at isoabsorpitive point.

➢ *Method III: Derivative Spectroscopy Determination:*

UV spectrum of both the drugs (ATV and FEN) were derivatised to first order with Δλ = 1 for the entire spectrum. Zero crossing points for ATV and FEN was found to be 239 and 246 nm respectively. Pure drug sample solutions were prepared as discussed above and the readings were taken in the first order mode at the selected wavelengths. Optical and regression data were tabulated in table 1.Accuracy of the method was checked by preparing five mixed standards containing different concentration, absorbance was measured at respective zero crossing points in first order UV spectrum and amount present in the sample was calculated from their respective calibration curve.

III. ANALYSIS OF TABLET FORMULATION

Twenty tablets were weighed accurately; the average weight was determined and then ground to fine powder. A quantity equivalent to 160mg of FEN was transferred to 100ml volumetric flask. Accurately weighed 150mg of ATV was transferred to same volumetric flask. , sufficient methanol was added sonicated for 5 min and diluted to the mark with same solvent. It was filtered through Whatman Filter paper no: 41, filtrate was suitably diluted to get final concentration with methanol. Absorbance's were measured at the 247,254 & 289nm wavelengths in the zero order and at the 239 & 246 nm in the first order, and amount present was calculated using simultaneous equation, absorbance correction and first order derivative methods. Findings are tabulated in table 2.

IV. RECOVERY STUDIES

To study accuracy, reproducibility and precision of the proposed methods, recovery studies were carried out by standard addition method. Results of recovery studies were found to be satisfactory and presented in Table 3. Precision of the method was determined by performing Intra Day (n = 3) and Inter Day (n = 3) refer the results in table 4.

V. RESULTS AND DISCUSSION

Three simple simultaneous estimation methods were successfully developed for the estimation of ATV and FEN in raw material and combined dosage form.

➢ *Linearity*

Calibration curves were prepared for both the drugs at the selected analytical wavelengths are summarized in Table1.This shows that ATV and FEN obeys Beer's law in the concentration range of 0-24 µg/ml.

➢ *Accuracy:*

The accuracy of the method was determined by investigating the recovery of ATV and FEN three levels ranging from 50, 75 &100% of the nominal concentration by standard addition technique. The results as shown in Table3 indicate excellent recoveries.

➢ *Precision &Repeatability:*

The precision and repeatability of the method was studied by repeating the proposed method three times in a day and the average percentage, RSD values of the results were tabulated, and when the experiment was repeated on three different days the average percentage RSD values for determination was tabulated in Table4. The results confirm the intra day and inter day precision of the method.

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VI. CONCLUSION

All the three method is a suitable for the reliable analysis for commercial formulations containing combinations of ATV and FEN. The methods are simple, precise, rapid and accurate. High percentage recovery shows that the method is free from the interference of excipients used in the formulation.

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Table 1 Optical Regression Characteristics of Both Drugs

	*ZERO ORDER						*FIRST ORDER	
Parameters	**247nm**		**289nm**		**254nm**		**239nm**	**246 nm**
	ATV	**FEN**	**ATV**	**FEN**	**ATV**	**FEN**	**ATV**	**FEN**
Beer's Law Limit (µg/ml)	0 -24	0 -24	0 -24	0 -24	0 -24	0 -24	0 -24	0 -24
Molar Absorptivity	24990.78	11154.68	10912.4629	21048.57	23182.31	14563.65	350.3201	540.4674
Regression Equation: Slope	0.044876	0.030661	0.01965	0.057887	0.041741	0.040174	0.00063	0.001489
Intercept	-0.00142	0.002524	-0.001196	0.004452	-0.00244	0.001863	-2.5E-05	8.85E-05
Correlation coefficient	0.999906	0.999682	0.99976087	0.999903	0.999593	0.999738	0.999915	0.999866
Sandell's Sensitivity	0.022404	0.032743	0.0514	0.017291	0.024013	0.02497	1.588833	0.675546
Standard error of mean	0.000762	0.000644	0.00080	0.000913	0.001603	0.00092	7.05E-05	0.000221

*Each reading is an average of six replicates

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Table 2 Results of Analysis of Formulation

S.No	Methods	Drug	Label Claimmg	% Label claim found*	±S.D	% RSD
1	Simultaneous	ATV	10	100.3269	0.24744	0.246634
	Equation	FEN	160	100.2635	0.185268	0.184781
2	Absorbance	ATV	10	100.1853	0.461863	0.461009
	Ratio	FEN	160	100.3347	0.308066	0.307038
3	First Order	ATV	10	101.147	0.99639	0.98509
	Derivative	FEN	160	100.5264	0.84879	0.84435

*Each Reading is an Average of Six Replicates

Table 3 Recovery Study

S.No	Method	% Recovery*		±S.D		% RSD	
		ATV	FEN	ATV	FEN	ATV	FEN
1	Simultaneous Equation	99.91 101.88 101.9	100.06 99.78 100.02	0.795 1.1 1.008	0.796 1.08 0.9898	0.628 0.144 0.074	0.628 0.145 0.07404
2	Absorbance Ratio	99.80 99.79 99.84	100.57 100.57 100.8	1.43 0.854 1.76	0.396 0.108 0.300	1.433 0.855 1.759	0.3936 0.0108 0.298
3	First order Derivative	99.90 99.90 100.28	99.86 99.67 100.03	0.037 0.947 1.032	0.9139 0.8215 0.8398	0.8379 0.948 1.0287	0.915 0.8242 0.8395

*Each reading is an average of six replicates

Table 4 Results of Intra Day & Inter Day Studies (N = 3)

S.No	Method	INTRA DAY %AMOUNT FOUND*		INTERDAY %AMOUNT FOUND*		% RSD1		% RSD2	
		ATV	FEN	ATV	FEN	ATV	FEN	ATV	FEN
1	Simultaneous Equation	99.45 100.93 101.18	100.55 99.88 100.46	99.45 101.44 100.71	100.5 98.91 99.69	0.691 1.0727 0.2298	0.691 0.643 0.714	0.69 0.64 1.054	0.70 0.204 0.855
2	Absorbance Ratio	99.43 99.67 99.1 4	101.06 100.9 101.2	99.25 99.49 99.43	101.25 101.00 101.00	0.89 1.08 1.029	0.886 1.00 1.02	1.15 0.96 0.86	1.16 0.948 0.915
3	First order Derivative	100.71 100.48 100.92	100.62 100.53 100.53	100.92 100.7 100.28	100.52 100.89 100.8	0.996 0.683 0.837	0.456 0.556 0.587	0.837 0.764 0.725	0.468 0.654 0.708

*Each reading is an average of six replicates

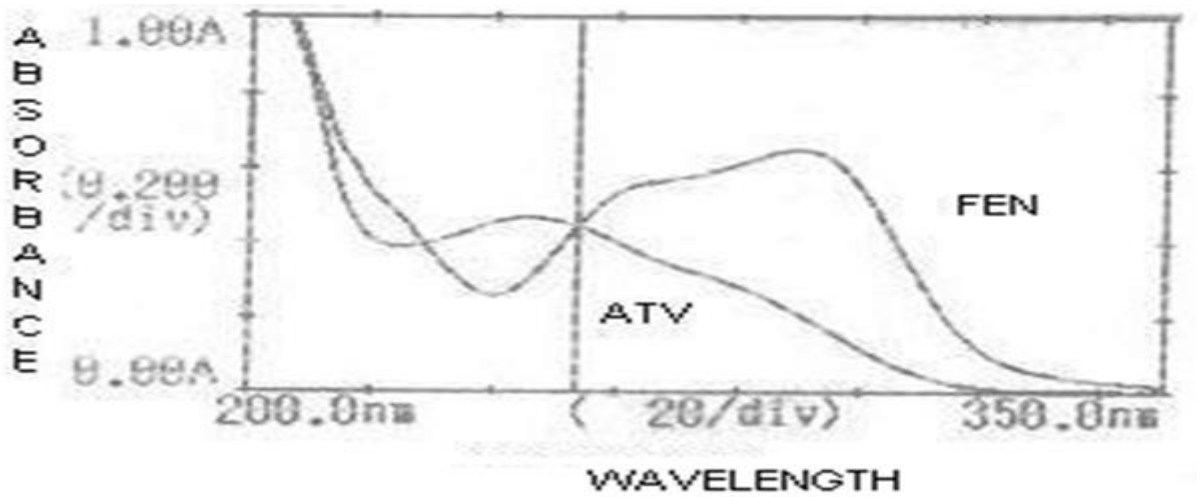

Fig 1 Overlain Spectra of Atrovastatin Calcium and Fenofibrate

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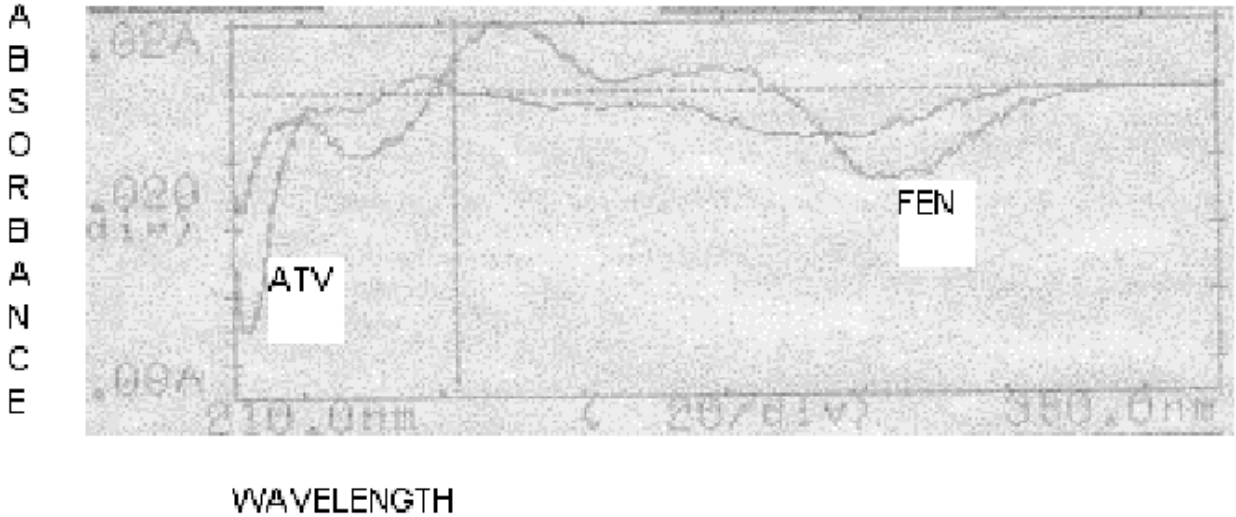

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