Simultaneous spectroscopic estimation and validation of atorvastatin calcium and pioglitazone in a tablet dosage form

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A specific, sensitive, precise and reproducible spectrophotometric method has been developed for the simultaneous estimation of Atorvastatin Calcium and Pioglitazone in tablet dosage form. An ultraviolet (UV) spectrophotometic method was developed and validated for quantitative determination of Atorvastatin Calcium and Pioglitazone in combined dosage form using methanol as solvent. The proposed method is based on simultaneous equation method. The absorption maxima (λ_{max}) of Atorvastatin and Pioglitzone were 245 and 226nm respectively. The calibration curves were prepared. The proposed method obeys Beers law in concentration range 5-35 µg/ml for Atorvastatin Calcium and Pioglitazone respectively with correlation coefficients were found to be 0.9960 and 0.9967 respectively. The method was contempt using known concentration of drug and percentage recovery was found in the range of 99 % to 101 %. Analyzing the combination in tablet dosage form, recovery study showed a good agreement in the assay of results. The proposed method is simple, precise, and accurate and can be employed for routine analysis of Atorvastatin Calcium and pioglitazone in tablet dosage form.

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1. Introduction

Atorvastatin (ATV), [(ßR,δS)-2-(4-fluorophenyl)- ß, δ-dihydroxy-5-(1-methyl ethyl)-3-phenyl-4[phenylamine]carbonyl]-1H-pyrrole-1-heptanoic acid calcium salt [1-3] is a lipid lowering agent acting through the inhibition of HMG-Co-A reductase. It is used in hypercholesterolemia; several methods for its estimation using HPLC[4-5] and HPTLC [6] are reported. Pioglitazone hydrochloride , Chemically [(±) -5-[[4-[2-(5-ethyl-2-Pyridinyl) ethoxy]phenyl]methyl]-2,4]thiazolidine-dione monohydrochloride, is thaizolidine-dione derivative that highly selective agonist for peroxisome proliferator – activated receptor gamma (PPAR) & is used as an adjunct to diet to improve glycemic control in patient with type 2 diabetes (non-insulin –dependent diabetes mellitus). The literature survey reveals the chromatographic methods are reported for simultaneous estimation of pioglitazone & its metabolites in human plasma, human serum, and urine.[7-12] Since Atorvastatin and Pioglitazone are marketed in combination and no simultaneous methods are reported for the estimation of these drugs in combined dosage form.

2. Experimental

UV/Visible spectrophotometer (Shimadzu Model 1601) was employed with spectral bandwidth of 1 nm and wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells). Analytical grade reagents and solvents were used for the study; pure sample of ATV and PIO was obtained as gift sample from Macleods Pharmaceutical Ltd Mumbai respectively. The tablet dosage form, PIAT (Label claim ATV 10 mg, PIO 10mg) by Cadila lalaboratory Ltd Ahmedabad were procured from local market. Methanol AR was obtained from Merck Limited, Mumbai, India.

3. UV-Spectrophotometry

3.1 Preparation of standard and sample solution

The stock solution was prepared by dissolving 100mg of Atorvastatin in 75 ml of Methanol in 100 ml volumetric flask, shaken and the volume was made up to the mark with Methanol,10 ml of this solution was diluted up to 100ml with Methanol in another volumetric flask produce final stock solution of 100 µg/ml of Atorvastatin. Standard stock solution of Pioglitazone was prepared similarly as that of Atorvastatin. Ten tablets each of two batches, batch A and batch B, Brand name PIAT manufactured by Cadila lalaboratory Ltd Ahmedabad were procured from local market. Their average weight was calculated. Ten tablets of each batch was crushed and weight equivalent to 100 mg of Atorvastatin was taken and dissolved in 75ml of Methanol with frequent shaking for 30 min. the final volume was made up to the mark (100ml) with Methanol. The sample solution was then filtered through Whatmann filter paper no 41 and first few ml were rejected. From two

solutions, 1 ml of the solution was taken and diluted to 10ml to get a stock solution containing 100µg/ml of Atorvastatin and corresponding concentration of Pioglitazone. Beer-Lambert's law was found to be obey in the concentration range of 0-10µg/ml for both the drugs in all the three methods. For method A and B five mixed standards solutions with concentration of ATV and PIO in µg/ml of 5:35,10:30,15:25,20:20,25:15,30:10,35:5 Overlain spectra of ATV and PIO were scanned (Fig. 1).

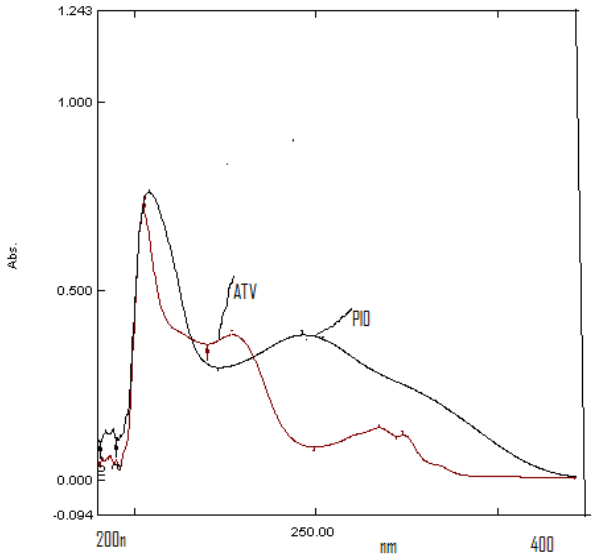


Fig. 1. Overlain spectra ATV and PIO.

2.2 Method A: simultaneous equation method

This method of analysis was based on the absorption of drugs (ATV and PIO) at the wavelength maximum of the each other [13]. Three wavelengths selected for the development of the simultaneous equations were 245 nm, 226 nm; λ max of all two drugs respectively. The absorptivity values E (1%, 1cm) were determined for three drugs at all selected wavelengths. The concentration of two drugs in mixture was calculated by using following equations.

The concentration of drugs in mixture can be calculated by using following equations:

$$C_{ATV} = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 - ax_1 ay_2} \quad (1)$$

$$C_{PIO} = \frac{A_1 ax_2 - A_2 ax_1}{ax_2 ay_1 - ax_1 ay_2} \quad (2)$$

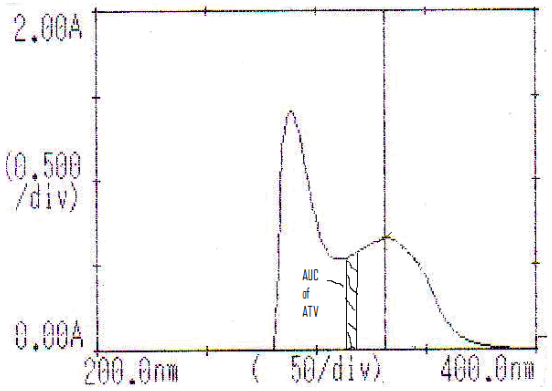
where, C_{ATV} and C_{PIO} are the concentration of ATV and PIO respectively in mixture and in sample solutions. (Table 1) A_1 and A_2 are the absorbances of sample at 245 nm and 226 nm respectively. ax_1 and ax_2 are the absorptivity of ATV at 245 nm and 226 nm respectively. ay_1 and ay_2 are the absorptivity of PIO 245 nm and 226 nm, respectively.

2.3 Calibration curve for ATV and PIO

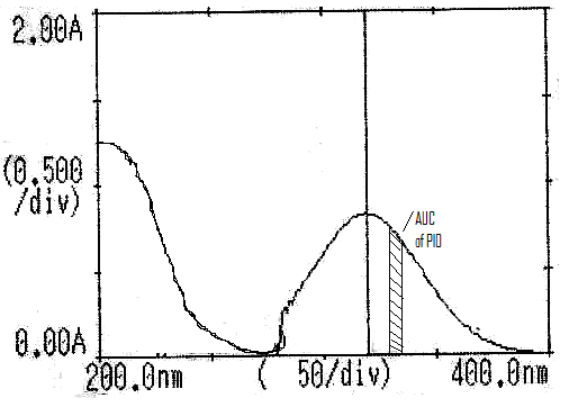
Absorbance of the solutions of the series A were measured at 245nm and absorbance of the solutions of series B were measured at 226 nm.Absorbance at 245 is due to ATV and plotted against ATV concentration (µg/ml) and absorbance at 226nm is due to PIO and plotted against PIO concentration (µg/ml)

2.4 Method B: derivative spectrophotometry method

In this method [14-15] 20µg/ml solution for both the drugs were prepared and scanned in the range of 400nm to 200nm. The spectra obtained were derivatized in first order and then recorded, which showed ATV had zero crossing point at 245 nm, while PIO had zero crossing point at 226nm (Fig. 2). At the zero crossing point of ATV, PIO showed a measurable dA/dλ where as at the zero crossing point of PIO, ATV showed appreciable dA/dλ. Hence both wavelengths 245 nm and 226 nm were selected as analytical wavelengths for estimation of ATV and PIO respectively. Calibration curves were plotted for ATV (5-35µg/ml) at 245 nm and PIO (5-35µg/ml) at 226 nm as dA/dλ v/s concentration.



(a)



(b)

Fig. 2. (a) area under curve ATV and PIO; (b) area under curve ATV and PIO.

2.5 Method C: area under curve method (AUC)

AUC method [16] involves the calculation of integrated value of absorbance with respect to wavelength. Area calculation processing item calculates the area of bounded by the curve and horizontal axis. Here horizontal axis represents baseline.

$$(\alpha+\beta)=\int_{\lambda_2}^{\lambda_1} Ad\lambda$$

where, α = area of portion bounded by curve data and a straight line connecting the start and end point, β= area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ_1 and λ_2 are wavelengths representing start and end point of curve region.

This method involved calculation of concentration for ATV in the regions of 264-260 nm and for PIO in the region of 297-294 nm, these regions were selected on the basis of repeated observation that plot area calculation of pure sample drug against the concentration. The UV spectra of ATV and PIO along with its AUC region are reported in Fig. 2 (a) and (b), respectively.

$$\int_{260}^{264} Ad\lambda = K_1C_1 \text{.....Eqn.1}$$

$$\int_{294}^{297} Ad\lambda = K_2C_2 \text{.....Eqn.2}$$

$$\int_{260}^{264} Ad\lambda = K_3C_1 \text{.....Eqn.3}$$

$$\int_{294}^{297} Ad\lambda = K_4C_2 \text{.....Eqn.4}$$

where C_1 and C_2 were concentration of ATV and PIO respectively in µg/ml and K_1, K2, K3 and K_4 were constant having values 0.2571, 0.1041, 0.1284 and 0.1738 respectively. Area of curve between 264-260 nm and 297-294 nm represented as $\int_{260}^{264} Ad\lambda$ and $\int_{294}^{297} Ad\lambda$ for ATV and PIO respectively. In view of that, following two final equations were developed for estimation of ATV and PIO.

$$\int_{260}^{264} Ad\lambda = K_1C_1 + K_2C_2 \text{.....Eqn.5}$$

$$\int_{294}^{297} Ad\lambda = K_3C_1 + K_4C_2 \text{.....Eqn.6}$$

Sample solutions were scanned and area was calculated within the indicated wavelength regions. Concentration of both components were calculated using Eqn. 5 and 6.

2.6 Validation of the developed methods

The developed methods for simultaneous estimation of ATV and PIO were validated as per ICH guidelines.

3. Accuracy

To check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments was carried out by standard addition method [17]. From that total amount of drug found and percentage recovery was calculated. The results and statistical data are reported in Table 1.

Table 1. Result of tablet dosage form containing ATV and PIO.

Parameters	Method-A		Method-B		Method-C	
	ATV	PIO	ATV	PIO	ATV	PIO
Label claim (mg/Tab)	10	10	10	10	10	10
Found (mg/Tab)	9.91	10.12	9.86	10.03	9.96	9.91
Drug content[a]	99.84	100.6	98.92	100.3	99.93	99.10
±S.D	0.834	0.257	0.643	0.851	0.268	0.386
%COV	0.370	0.854	0.244	0.922	0.669	0.287
SE	0.241	0.391	0.263	0.388	0.428	0.308

[a]*Value for drug content (%) are the mean of five estimation, Method-A: Simultaneous equation method, Method-B:, Derivative spectrophotometry method Method-C: Area under curve method , S.D: Standard deviation , COV: Coefficient of variance and S.E : Standard error. ATV and PIO denote Atorvastatin Calcium and Pioglitazone, respectively.*

4. Precision

4.1 Repeatability

To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Five samples of the tablet formulations were analyzed for the repeatability study. The standard deviation, coefficient of variance and standard error was calculated. The results were reported in Table 1.

4.2 Intermediate precision (inter-day and intra-day precision)

The intra and inter-day precision was calculated by assay of the sample solution on the same day and on

different days at different time intervals respectively. The results are presented in Table 2.

Table 2. Intraday, Interdays, LOD and LOQ data of tablet formulation.

Method	Drug	Intra day precision %COV (n =3)	Interday precision %COV		
			Day 1[a]	Day 2[a]	Day 3[a]
Method A	ATV	0.23	0.315	0.217	0.163
	PIO	0.128	0.612	0.318	0.286
Method B	ATV	0.29	0.190	0.101	0.115
	PIO	0.182	0.145	0.214	0.332
Method C	ATV	0.312	0.052	0.161	0.215
	PIO	0.193	0.219	0.316	0.392

[a]*Mean of five determinations, COV: Coefficient of variance*

4.3 Analysis of combined dosage form

The absorbance of final sample solution was measured against methanol as blank at 245 and 226nm. The amount of ATV and PIO was computed by adding the absorbance value in simultaneous equation.

4.4 Recovery study

The method was validated by recovery study were carried out by the addition of different amount of drugs to pre analyze solution (10µg/ml). From the stock solution of 100µg/ml of each drug 1ml solution was taken in each of four volumetric flask (10ml), then 1.2, 0.8, 0.4 ml of mixed standard stock solution (100µg/ml of Atovastatin and 100µg/ml of Pioglitazone) added in three flasks so that remaining one flask contains no added solution. These solutions were scanned at 245nm and 226nm. Percentage recovery was found in the range of 99% to 101%. Table 3.

Table 3. Result of recovery studies.

Methods	Drug	Label claim (mg/tab)	Amount Added at	%Recovery ±S.D	% COV
Method-A	ATV	10	80	100.01	0.3081
			100	99.96	0.0507
			120	100.02	0.1050
	PIO	10	80	100.04	0.2713
			100	100.03	0.2418
			120	99.97	0.3124
Method-B	ATV	10	80	99.9	0.2153
			100	99.88	0.3057
			120	99.68	0.8613
	PIO	10	80	99.65	0.2145
			100	99.89	0.0563
			120	99.96	0.2254
Method-C	ATV	10	80	100.04	0.0328
			100	100.05	0.3301
			120	99.96	0.1625
	PIO	10	80	100.01	0.0221
			100	99.70	0.4150
			120	100.03	0.1618

Recovery is mean of Two estimation, Method-A: Simultenous equation Method- B: Derivative spectrophotometry method, Method-C: Area under curve method, S.D: Standard deviation and COV: Coefficient of variance.

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