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Micellar Liquid Chromatographic Analytical Method Development and Validation of Determination of Atorvastatin Calcium and Pioglitazone in Tablet Dosage Form

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Abstract

A simple rapid, simple and sensitive liquid chromatographic procedure that use micellar mobile phase containing only Tween-20 and n-Butanol, is reported for the determination of method for estimation of method has been developed and validated for simultaneous estimation Atorvastatin Calcium (ATV) and Pioglitazone (PIO) in tablet dosage form. The estimation was carried out on Luna C_{18} (5µm×25cm×4.6mm) column with a mixture of Tween-20 and n-Butanol Phosphate buffer, pH 4.2 (50:25:25v/v) at flow rate of 1.5 ml/min at 25^{o}C temperature. Quantitation was achieved by UV detection at 322 nm over lain spectra (figure-3) the concentration range 5-210 mg/ml for both the drugs with mean recoveries of 99.01% $\pm$ 0.12 and 100.64% $\pm$ 0.20 for ATV and PIO respectively. This method is simple, precise and sensitive and it is applicable for the simultaneous estimation of ATV and PIO in tablet dosage form.

Key Words: Atorvastatin Calcium, Pioglitazone HCl, Micellar liquid chromatography, Tween-20.

Introduction

Micellar solution can replace conventional aqueous organic mobile phase with good results. Micellar liquid chromatography (MLC) is a reversed phase liquid chromatographic (RPLC) mode with mobile phases containing a surfactant (Ionic or Non ionic) above its critical concentration (CMC) [1]. In these conditions the stationary phase is modified with an approximately constant amount of surfactants monomers, and solubilizing capability of mobile phase is altered by the presence of micelles, giving rise to diverse interactions (Hydrophobic, ionic and satiric) with major implications and selectivity. Literature survey

revealed that no HPLC method has been reported for the estimation of in combined dosage form.Because of the absence of an official pharmacopoeial method for the Micellar liquid chromatography method of ATV and PIO in tablet dosage form; efforts were made to develop an analytical method for the estimation of ATV and PIO in tablet dosage form using HPLC method. Micellar mobile phases have been used with different bonded stationary phases (mostly C8, C18 and cyanopropyle). The most common surfactant is the anionic sodium dodecyl sulphate (SDS) cationic cetytrimethylammonium bromide (CTAB), and non-ionic Tween-20. Several organic solvents have been used as modifiers, short or medium chain alcohols and acetonitrile being the most suitable. The presence of micellar contributes well above their solubility in water also the risk of evaporation is diminished.

Atorvastatin (ATV), [(ßR, δS)-2-(4-fluorophenyl)-ß, δ-dihydroxy-5-(1-methylethyl)-3-phenyl 4[phenylamine] carbonyl]-1H-pyrrole-1heptanoic acid calcium salt [2-6] 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 [7-8] and HPTLC [9] 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) and 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 and its metabolites in human plasma, human serum, and urine [10-14]. Since Atorvastatin and Pioglitazone are marketed in combination and no RP-HPLC simultaneous methods are reported for the estimation of these drugs in combined dosage form. Because of the absence of an official pharmacopoeial method for the simultaneous estimation of ATV and PIO in tablet dosage form, efforts were made to develop an analytical method for the estimation of ATV and PIO in tablet dosage form using HPLC method.

Results and Discussion

To optimize the HPLC parameters, several mobile phase compositions were tried. A satisfactory separation of ATV and PIO with good peak symmetry and steady baseline was obtained with mobile phase Tween-20, n-Butanol Phosphate buffer (50:25:25 v/v) adjusted to pH 4.2. Quantization was achieved with UV detection at 322 nm based on peak area. Complete resolution of the peaks with clear baseline separation was obtained (Figure 1).The system suitability test parameters are shown in (Table 1).

Validation of the proposed method

Linearity

Linear correlation was obtained between peak areas and concentration of ATV and PIO in the range of 5-25µg/ml for both the drugs, respectively. Data of the regression analysis are summarized in (Table 3).

Accuracy

The recovery experiments were performed by standard addition method. The recoveries obtained were 100.12 $\pm$ 0.10 % and 99.98 $\pm$ 0.11% for ATV and PIO respectively (Table 4).

Method precision

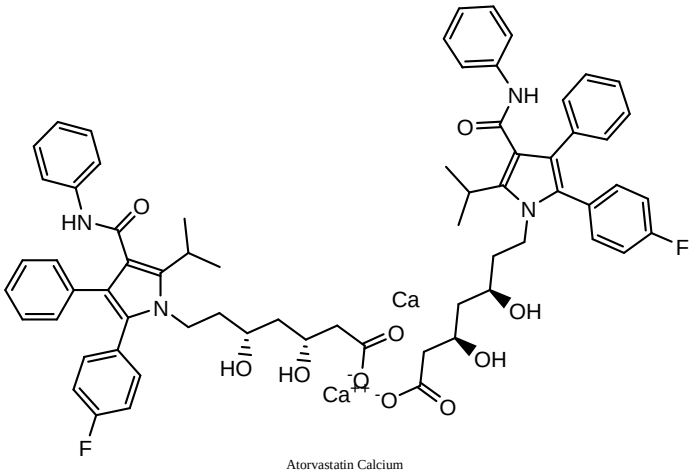
The RSD values for ATV and PIO were found to be 0.54 % and 0.25 % respectively (Table 4).

Intermediate precision

The RSD values were found to be < 1%, which indicates that the proposed method is reproducible (Table 4).LOD and LOQ – LOD values for ATV and PIO were found to be 0.02 and 0.004µg/ml respectively. LOQ values for ATV and PIO were found to be 0.01 and 0.07 µg/ml respectively (Table 4).

Assay of the tablet dosage form (ATV 10 mg/tablet and PIO 10 mg/tablet)

The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard Solution. The proposed validated method was successfully applied to determine ATV and PIO in tablet dosage form. The result obtained for ATV and PIO were comparable with corresponding labeled amounts (Table 5).



Atorvastatin Calcium

Figure-1 Atorvastatin Calcium Molecule

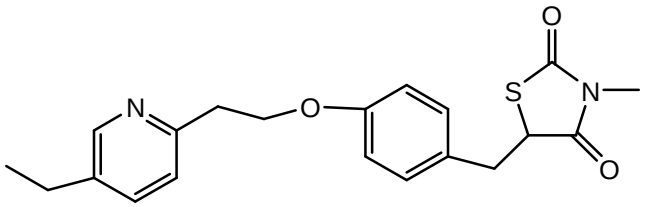


Figure-2 Pioglitazone Molecule

Table1.System suitability test parameter for Atorvastatin Calcium and Pioglitazone

Property (n*=6)	ATV	PIO
Retention time(min)	6.943	9.946
Tailing factor ATV	3.24	1.32
Capacity factor ATV	1.443	3.765
Theoretical plates number	4321	6532
Resolution	2.65	4.34

* n = Number of determination, ATV- Atorvastatin Calcium, PIO-Pioglitazone

Table 2 Recovery Study Atorvastatin Calcium and Pioglitazone

ATV				PIO			
Label claimed	% Amount added	Found in(µg/ml)	% recovery	Label claimed	% Amount added	Found in(µg/ml)	% recovery
10	80	10.11	100.01	10	80	9.98	99.97
	100	10.01	100.06		100	10.09	99.88
	120	99.98	99.97		120	9.99	100.03

Table 3 Regression Analysis of Calibration Graph for Atorvastatin Calcium and Pioglitazone

Parameter	**ATV**	**PIO**
Concentration range	5-25 µg/ml	5-25 µg/ml
Slope	18243	26736
SD[$] of the slope	3.85	5.75
Intercept	657474	896753
SD[a] of the intercept	6.768	12.763
Correlation coefficient	0.9998	0.9999

[$] SD = Standard Deviation ATV- Atorvastatin Calcium, PIO-Pioglitazone

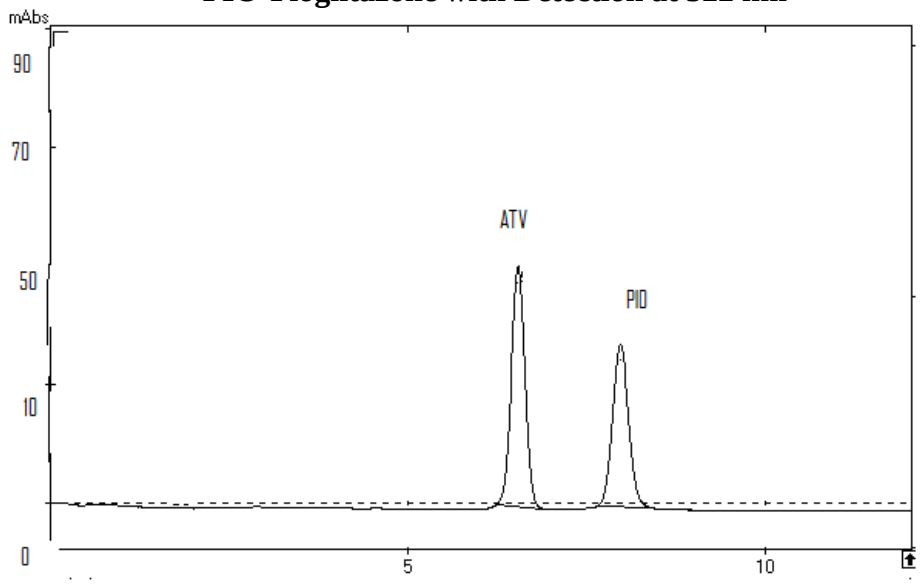
Table 4 Summary of validation parameter Atorvastatin Calcium and Pioglitazone

Parameter	**ATV**	**PIO**
LOD[a]	0.09µg/ml	0.07µg/ml
LOQ[b]	0.02µg/ml	0.03µg/ml
Accuracy, %	99.98 ± 0.54	100 ± 0.11%
Repeatability(RSD[c] %, n =6)	0.543	0.321
Precision (RSD, %)		
Intraday (n =3)	0.055	0.046
Interday (n = 3)	0.035	0.065

Table 5- Result of Assay of Tablet Formulation Atorvastatin Calcium and Pioglitazone

ATV		**PIO**	
Amount claimed (mg/tablet)	Amount found (mg/tablet)	Amount claimed (mg/tablet)	Amount found (mg/tablet)
10	10.08	10	9.95
	9.97		10.03
	10.05		9.97
	9.97		10.02
	10.01		10.01
	10.21		9.92
Mean	1.432	Mean	2.758
±SD	0.325	±SD	0.121

Figure-3 High Performance Liquid Chromatogram of ATV- Atorvastatin Calcium, PIO-Pioglitazone with Detection at 322 nm



Materials and Methods

Apparatus

High performance liquid chromatograph, Shimadzu pump LC-10ATVP equipped with Rheodyne injects ATV with 20µl fixed loop, Photo Diode Array detec ATV. SPD-MXA software was used.

Materials

ATV and PIO pure powder were procured as gifts sample from Sun Pharmaceutical Industries Silvassa Dadra and Nagar Hawali India. The tablet dosage form, PIAT (Label claim ATV 10 mg, PIO 10mg) by Cadila Ltd Ahmedabad were procured from local market. Tween-20, n-Butanol and water were obtained from Merck. All reagents were of HPLC grade unless otherwise specified. from E.Merck (Mumbai, India), Potassium Dihydrogen Phosphate and o- phosphoric acid were purchased from SD fine chemical Ltd (Ahmedabad, India) and were of analytical grade Water of HPLC grade was used. Potassium Dihydrogen Phosphate and o- phosphoric acid were purchased from SD fine chemical Ltd (Ahmedabad, India) and were of analytical grade Water of HPLC grade was used.

HPLC Conditions

The LicATVphere C_{18} column was used 25^{o}C temperature. The mobile phase considered the mobile phase considered 5% n-Butanol in 0.05 molL^{-1} ,Tween-20, pH adjusted to 4.2 $\pm$ 0.01 with o-phosphoric acid. It was pumped at flow rate of 1ml/min. the mobile phase was passed through nylon 0.45 µm membrane filters and degassed before use. The elution was moni ATV at 322 nm and the injection volume was 20 µl.

Preparation of standard stock solution

The equivalent of 10 mg each of ATV and PIO were accurately weighed in 100 ml volumetric flasks separately and dissolve in 25 ml of n-Butanol. After the immediate

dissolution, the volume was made up to the mark with solvent. These standard stock solutions were observed to contain 100 µg/ml of ATV and PIO

Preparation of sample solution

20 tablets were taken and their average weight was determined, they were crushed to fine powder. Then powder equivalent to 10 mg of ATV was taken in 25ml volumetric flask and dissolved in 75ml of methanol with vigorous shaking for 5-10 minutes. The supernatant liquid was transferred to 50ml of volumetric flask through a whattman # 41 filter paper. The residue was washed twice with solvent and the combined filtrate was made up to 100ml mark. After that 10 ml of the above solution was diluted up to 100 ml with solvent. The two main advantages of micellar procedure are the elimination of organic solvents and simplification of sample preparation step. The seven point's calibration graphs were constructed covering a concentration range. 0.5 to 5 mg/ml. linear relationship was obtained between the peak area ratio of ATV and PIO in the concentration range 35 ppm to 76 ppm. The correlation coefficient was found 0.9997. According to International Conference on Harmonization (ICH) guidelines the following expression is used to evaluate LOD and LOQ.

Method Validation

Linearity

Calibration graphs were constructed by plotting peak area Vs concentration of ATV and PIO and the regression equation were calculated. The calibration graphs were plotted over 5 different concentrations in the range of 5-25µg/ml for both drugs. Accurately measured mixed standard solution aliquots of ATV and PIO (0.5, 1.0, 1.5, 2.0, 2.5 ml) were transferred to series of 10 ml volumetric flasks and diluted to mark with methanol. Aliquots (20µl) of each solution were injected under the operating chromatographic condition described above [Number of replicates (n=6)].

Accuracy

The accuracy of the method was established using recovery technique i.e. external standard addition method. The known amount of standard was added at three different levels to preanalysed sample. Each determination was performed in triplicate. The result of recovery study is presented in (table 2).

Method precision (repeatability)

The precision of the instrument was checked by repeatedly injecting (n = 6) mixed standard solution of ATV and PIO.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing mixed standard solution of ATV and PIO at concentration 5µg/ml and 25µg/ml 3 times on the same day and on 3 different days. The results are reported in terms of relative standard deviation.

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

The LOD with signal to noise (S/N) ratio of 6:2 and LOQ with (S/N) ratio of 2:1 were calculated for both drugs using the following equations according to International Conference on Harmonization guidelines [15].

$$LOD = 8 \times \sigma/S$$
$$LOQ = 4.5 \times \sigma/S$$

Where σ = the standard deviation (SD) of the response and S = the SD of the y-intercept of the regression line.

Analysis of Atorvastatin Calcium (ATV) and Pioglitazone (PIO) in tablet dosage form

The response of sample solutions were measured at 322 nm for quantitation of ATV and PIO by the method described above. The amount of ATV and PIO present in the sample solution were determined by applying values of peak area to regression equation of the calibration graph.

Assay of Tablet Formulation

Before assay of the tablet formulation five replicate of required dilutions were prepared from the tablet stock solution and sonicated for 10 min. After that 20µl of the solution was injected for quantitative analysis. The amounts of ATV and PIO per tablet were calculated by extrapolating the value of area from the calibration curve.

Validation

The method was validated for linearity, accuracy, precision, repeatability, selectivity and specificity. Accuracy study was carried out by doing recovery study as per ICH norms at three different levels- 80%, 100%, and 120%. Precision was carried out by doing intra and inter day precision study. In intra day study concentration of both drugs were calculated for three times on the same day at an interval of one hour. In inter day study the concentration of drug contents were calculated on three different days. Selectivity and specificity of the method was validated by injecting solutions containing both the drugs and after running two sharp peaks were obtained for both drugs. LOD and LOQ study was carried out to evaluate the detection and quantitation limits of the method to determine presence of any impurities.

Conclusion

The proposed miceller chromatographic method has been evaluated over the linearity, precision, accuracy, specificity and proved to be conveninant and effective for the quality control The proposed method has advantage of simplicity and convenience for the separation and quantitation of ATV and PIO in the combination and cab be used for the assay of their dosage form. Also, the low solvent consumption and short analytical run time lead to environmentally friendly chromatographic procedure. As the result shows that the method could find practical application hence, utilized as quality control tool for the simultaneous estimation of both drug from their combined dosage form in quality control laboratory. The method is accurate, precise, rapid and selective for simultaneous estimation of Atorvastatin Calcium and Pioglitazone in tablet dosage form. Hence it can be conveniently adopted for routine analysis.

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