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HPTLC method development and validation for the estimation of Atorvastatin Calcium and Pioglitazone Hydrochloride in pharmaceutical combined tablet dosage form

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Abstract

A new simple, accurate and precise HPTLC method has been developed for the simultaneous estimation of Atorvastatin Calcium (ATV) and Pioglitazone (PIO) in tablet dosage formulation. In this method, standard and sample solutions of Atorvastatin Calcium and Pioglitazone in tablet dosage form were applied on the stationary phase used was precoated silica gel 60 F under 254. The mobile phase used was a mixture of chloroform: methanol: toluene (6:3:4 v/v). The detection of spot was carried out at 259 nm. The calibration curve was found to be linear between 100 to 400 ng/spot for Atorvastatin Calcium and Pioglitazone. The proposed method can be used to determine the drug content of marketed formulations. The method was validated for precision, accuracy, and reproducibility.

Key Words: Atorvastatin Calcium, Pioglitazone Hydrochloride, HPTLC.

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]. No HPTLC method was found reported for the simultaneous estimation of the drugs. In the present article, we report a simple and accurate high performance thin layer chromatographic method for simultaneous estimation of Atorvastatin Calcium and pioglitazone from tablets.

Materials and Methods

Materials and Sample preparation

The instrument used for the estimation, was Camag Linomat V semi automatic sample applicator, Camag TLC scanner 3, CATS software for interpretation of the data, Hamilton syringe and Camag twin trough chamber.

ATV and PIO pure powder were procured as gifts sample from Sun pharma Dadra. The tablet dosage form, PIAT (Label claim ATV 10 mg, PIO 10mg) by Cadila Ltd Ahmedabad were procured from local market. The pre coated silica gel G 60 F 254 was used as stationary phase, obtained from E. Merck. All the solvents used were of AR grade, obtained form S. D. Fine Chemicals Ltd., Mumbai. The mobile phase used was a mixture of chloroform: methanol: toluene (6:3:4 v/v).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 methanol. 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.Twenty 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 75 ml of methanol with vigorous shaking for 25 minutes. The supernatant liquid was transferred to 50ml of volumetric flask through whatman no 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.

Results and Discussion

The TLC plates were pre-washed with methanol, and activated by keeping at 95 ^{o} for about 30 min. The drugs were well resolved on the precoated silica gel G 60 F_{254} on aluminum sheets, the mobile phase was chloroform: methanol: toluene (6:3:4 v/v/v), chamber saturation time 20 min, distance 30 mm, wavelength scanning at 259 nm, band width 9 mm, slit dimension keeping the slit dimension at 3 ×0.45 mm scanning speed 12 nm/sec, and the source of radiation of deuterium lamp. On to a pre-washed and activated TLC plate, 2-10 ml of standard stock solution of ATV and PIO was spotted with Linomat V Semi applicator. The plates were developed and scanned. The peak areas of each standard were obtained from the system, and a calibration graph was plotted with concentration vs. peak area. The method was validated for linearity, accuracy, limit of detection, limit of quantification, inter-day and intra - day assay precision, repeatability of measurement, and repeatability of sample application. From the sample aliquot prepared, 2 and 6 ml solution was applied, and the plate was developed with the mobile phase. A triplicate of those was carried out, and the peak areas were noted. The amount of ATV and PIO present in the formulation was calculated using the respective calibration graph.
To develop a precise, accurate and suitable HPTLC method for the quantitative determination of

ATV and PIO different solvent systems were employed and the proposed chromatographic condition was found appropriate for the quantitative determination. The mobile phase consisted of chloroform: methanol: toluene (6:3:4, v/v) and Rf value of ATV and PIO were found to be 0.45 and 0.30 respectively. Detection was carried out at 241, 259 ATV and PIO respectively. The proposed method has been validated for assay of ATV and PIO in bulk and tablet dosage forms using following parameters [13], [14]. The target analyte concentration of all the two drugs was fixed as 50 µg/ml. Linear calibration plots were obtained over the calibration ranges tested, i.e., 100 to 1000 ng/spot, 100 to 400 ng/spot ATV and PIO, respectively. The corresponding linear regression equations, with correlation coefficient ≥0.001, were y=1.3821x+321.24; y=2.8043x+231.43, ATV and PIO, respectively. Linearity was checked for three consecutive days for the same concentration range from the same stock solutions.Accuracy of the method was checked by recovery study using standard addition method, [Table-2] known amount of standard ATV and PIO were added into pre analyzed samples separately and subjected them to the proposed HPTLC method. These studies were carried out at three levels i.e., multiple level recovery studies. The intra- and inter-day precision were carried out at three different concentration levels, i.e., 100,300,500 ng/spot; 200, 400, 600 ng/spot for the determinations of ATV and PIO, respectively. The low values of percentage relative standard deviation (% RSD) for intra-and inter-day variation as shown in [Table-3] reveal that the proposed method is precise.

Table 1 Regression Analysis of Calibration Graph for ATV and PIO

Parameter	**ATV**	**PIO**
R_F (SD)	0.30	0.45
Linearity and range (ng\spot)	100	500
Linearity detection (ng\spot)	95	80
Limit of quantification (ng\spot)	241	259
Repeatability of application(%RSD)	0.20	0.55
Repeatability of measurement (%RSD)	0.90	0.75
Intraday (%RSD)	0.32	0.27
Inter day (%RSD)	0.15	0.22
LOD[a]	25.90	65.04
LOQ[b]	15.71	23.14

$ SD = Standard Deviation

Table 2 -Recovery Study

ATV				PIO			
Label claimed	%Amount added	Found in(µg/ml)	%recovery	Label claimed	%Amount added	Found in(µg/ml)	%recovery
10	80	9.98	98.93	10	80	10.03	100.05
	100	10.02	100.02		100	10.01	100.02
	120	10.06	100.05		120	9.97	99.93

Table 3 Result of Assay of Tablet Formulation

ATV		PIO	
Amount claimed (mg/tablet)	Amount found (mg/tablet)	Amount claimed (mg/tablet)	Amount found (mg/tablet)
10	9.99	10	9.99
	10.03		9.95
	9.99		10.07
	9.97		9.92
	10.02		9.85
	10.04		10.01
Mean	3.692	Mean	2.904
±SD	0.0381	±SD	0.0431

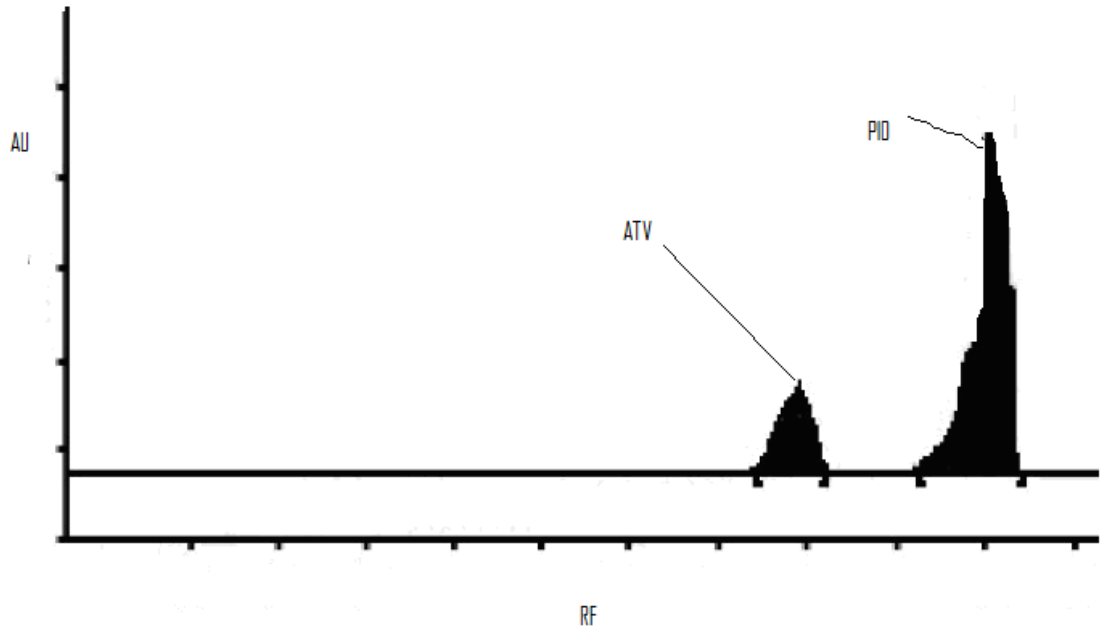

Fig.1 High Performance Thin Liquid Chromatogram of ATV and PIO

Method Validation

For ruggedness, study was carried out for two different parameters i.e., days and analyst. The results of estimation by proposed method are very much similar under variety of conditions. The assay results of ATV and PIO in bulk and tablet dosage forms were comparable with the value of labeled claim. The obtained results are given in [Table].

To study the accuracy of the proposed method recovery studies were carried out using standard addition method. The percent recovery was calculated by using the formula, % recovery= (T-A)/S×100, where T is total amount of drug estimated, A is the amount of drug contributed by tablet powder and S is the amount of pure drug added.

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 100,300,500 ng/spot; 200, 400, 600 ng/spot three times on the same day and on three different days. The results are reported in terms of relative standard deviation.

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

The limit of quantification (LOQ) represents the concentration of the analyte that would yield a signal-to-noise ratio of 10 [7]. The LOD and LOQ were found to be 100 and 250 ng/spot, respectively for ATV 9 and PIO 100 ng/spot,

Conclusion

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. The proposed method has advantage of simplicity and convenience for the separation and quantitation of ATV and PIO in the combination and can 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

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