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A Quantitative Estimation and Validation of Atorvastatin calcium and Pioglitazone in Tablet Dosage Form by Hydrotropic Solubilization Phenomenon

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Abstract: Various organic solvents like methanol, chloroform, alcohol, dimethyl formamide; acetonitrile, hexane, acetone and carbon tetrachloride have been employed for solubilization of poorly water-soluble drugs for spectrophotometric estimations. Drawbacks of organic solvents include higher cost, toxicity and error in analysis due to volatility. Attempting to minimize these drawbacks, three new, simple, accurate, environmental friendly, cost effective, safe, sensitive spectrophotometric methods have been developed for simultaneous estimation of Atorvastatin Calcium (ATV) and Pioglitazone (PIO) in tablet dosage form tablet dosage form using 8.0 M sodium benzoate aqueous solution, as a hydrotropic agent. Aqueous solubility of these model drugs were enhanced to a great extent 55 and 71 fold for Atorvastatin Calcium (ATV) and Pioglitazone (PIO) in 8.0M sodium benzoate solution respectively. Sodium benzoate solution and additives of tablet did not interfere in analysis, as sodium benzoate did not show any absorbance above 300nm. In 8.0M sodium benzoate solution, Atorvastatin Calcium (ATV) and Pioglitazone (PIO) in tablet dosage form shows maximum absorbance at wavelength 313.0 nm and 324 nm respectively and isobestic point was observed at 303 nm. Beer's law was obeyed in the concentration range 5-35 µg/ml for Atorvastatin Calcium and 5-40µg/ml for Pioglitazone. Method-I is based on simultaneous equation method Results of analysis for methods were tested and validated for various parameters according to ICH guidelines, hence can be adopted for the routine analysis of Atorvastatin Calcium (ATV) and Pioglitazone (PIO) in tablet dosage form.
Key Words: Atorvastatin Calcium, Pioglitazone HCl, Hydrotropic Solubilization, Simultaneous equation method.

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 [1-5]. 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 [6-11]. Various techniques have been employed to enhance the aqueous solubility of poorly water soluble drugs such as alteration in pH of solvent, co-solvents, complexation, hydrotropic solubilization etc. The term "hydrotrophy" originally put forwarded by Newberg[12] to describe the increase in the solubility of the solute

by the addition of fairly high concentration of alkali metal salts of various organic acids. According to Newberg, the solublizing agent, are anionic organic salts. Saleh and El-Khordagui[13] made an attempt to extent the definition of the term hydrotropic agent to included cationic and nonionic organic compounds bearing the essential structural features of Newberg's hydrotropes. Cationic compounds such as p-amino benzoic acid hydrochloride, procaine hydrochloride and neutral molecules such as resorcinol and pyrogallol confer typical hydrotropic properties.Winsor[14] considered hydrotrophy as a solubilization phenomenon, with the solute dissolved in oriented clusters are the hydrotropic agents. Sodium salicylate[15], sodium benzoate, sodium lauryl sulphate[16], sodium glycinate, sodium gentisate, nicotinamide[17], urea[18] sodium acetate, sodium citrate[19] and, niacinamide[20] have been employed as a hydrotropic agent. A successful attempt have been made by using this phenomenon for the analysis of various poorly water soluble drugs viz. frusemide[21], cefixime[22], hydrochlorothiazide[23], ketoprofen[24], bulk sample of ketoprofen and salicylic acid[25], norfoxacin in combination with tinidazole[26] and metronidazole[27].Since Atorvastatin and Pioglitazone are marketed in combination and no hydrotrophy as a solubilization phenomenon 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 hydrotrophy as a solubilization phenomenon method.

EXPERIMENTAL

UV-visible double beam spectrophotometer, Shimadzu model 1700 with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells was used. 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.

Preliminary solubility studies of drugs

Solubility of both drugs was determined at 32 ±1°C. An excess amount of drug was added to three screw capped 30ml glass vials containing different aqueous system viz. distilled water, buffer of pH 7.7, 8.0M Sodium benzoate solution. The vials were shaken for 22 hrs at 32±1°C in a mechanical shaker. These solutions were allowed to equilibrate for the next 24 hrs and then centrifuged for 15 minutes at 2000rpm. The supernatant of each vial was filtered through Whatman filter paper No.41. The filtrates were diluted suitably and analyzed spectrophotometrically against corresponding solvent blank.

Preparation of standard stock, calibration curve and binary mixture solutions

The standard stock solutions of ATV and PIO were prepared by dissolving 50mg of each drug in 40ml of 8.0M sodium benzoate solutions and final volume was adjusted with distilled water in 100ml of volumetric flask. From the above solution 10ml of solution was taken and diluted to 50ml with distilled water to get a solution containing 100 µg/ml of each drug. Working standard solutions were scanned in the entire UV range of 400-200 nm to determine the λ max of both drugs. The λ max of ATV and PIO were found to be 313 nm and 324 nm respectively and from overlain spectra (Fig.1) it is evident that isobestic point was obtained at 287 nm. Eight working standard solutions for both drugs having concentration 5, 10, 15, 20, 25, 30, 35, 40µg/ml were prepared in distilled water from stock solution. The absorbances of resulting solutions for both drugs were measured at 313, 324 nm for method, and plotted a calibration curve against concentration to get the linearity and regression equation of both drugs. Six mixed standards solutions with concentration of ATV and PIO in µg/ml of 30:5,25:10,20:15,15:20,10:25,5:30 were prepared in distilled water by diluting appropriate volumes of the standard stock solutions.

Method- (Simultaneous equation method)

Simultaneous equation method[28] of analysis was based on the absorption of drugs (ATV and PIO) at the wavelength maximum of the each other. Two wavelengths were selected for the development of the simultaneous equations was 313 nm and 324 nm, λmax of ATV and PIO respectively. The absorbances of both the drugs were measured at 313 nm and 324 nm. The absorptivity values E (1%, 1cm) determined for ATV at 313 nm and 324 nm were 281(ay_2) and 298 (ay_1) while respective values for PIO were 264 (ax_2) and 331 (ax_1). These values were means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in following equations to obtain the concentration of both drugs.

$$CATV = \frac{(A_1 x 331) - (A_2 X 264)}{-234302} \quad \text{.....Eqn.1}$$

$$C_{PIO} = \frac{(A_2 x 264) - (A_1 x 281)}{-154676} \quad \text{.....Eqn.2}$$

C_{ATV} and C_{PIO} are the concentration of ATV and PIO respectively in mixture and in sample solutions. A_1 and A_2 are the absorbances of sample at 313 nm and 324

nm respectively. ax_1 and ax_2 are the absorptivity of ATV at 313 nm and 324 nm respectively. ay_1 and ay_2 are the absorptivity of PIO 245 nm and 226 nm respectively.

$A_1 = 331xCATV + 298xC_{PIO}$ and,

$A_2 = 281xC_{ATV} + 264xC_{PIO}$

Analysis of the tablet formulations

Twenty tablets of marketed formulation were accurately weighed and powdered. A quantity of powder equivalent to 50 mg of ATV was transferred to 100 ml volumetric flask and dissolved in 40 ml of 8.0M sodium benzoate with frequent shaking for 15minutes and final volume was made up with distilled water. The sample solution was then filtered through Whatman filter paper No.41 and first few ml were rejected. From the above solution 10ml of solution was taken and diluted to 50ml with distilled water to get a solution containing 100 µg/ml of ATV and corresponding concentration of PIO. This solution contains ATV and PIO in the proportions of 1:2.5. 2.0 ml of solution was transferred in 10ml volumetric flask and diluted with distilled water to obtain final concentration of 5 µg/ml of ATV and 18 µg/ml of PIO. For method-I absorbance of the sample solution viz. A_1 and A_2 were recorded at 313 and 324 nm respectively and concentration of two drugs in the sample were determined using Eqn.1 and 2.

Table 1: Optical Characteristics data of Atorvastatin Calcium and Pioglitazone

Parameters	Values	
	ATV	PIO
Working λ (nm) in 8.0M sodium benzoate solution	313	324
Beer's law limit (µg/ml)	5-35	5-40
Absorptive E (1%, 1cm)*	281	298
Correlation coefficient*	0.9998	0.9999
Intercept*	0.0002	0.0013
Slope*	0.0288	0.0031

ATV: Atorvastatin Calcium, PIO: Pioglitazone, *Average of six estimation

Table 2: Analysis Data of Tablet Formulation, Statistical Validation and Recovery studies

Method	Drug	Label claim mg/tab	Amount found* mg/tab	Label claim (%)	S.D.*	% COV	S.E.*	Amount added at (%)	% Recovery #
I	ATV	10	10.02	100.11	0.213	0.432	0.121	80	99.98
								100	99.91
								120	100.08
	PIO	10	10.0	100.02	0.173	0.2134	0.328	80	98.95
								100	98.75
								120	100.05

ATV: Atorvastatin Calcium, PIO: Pioglitazone S.D.: Standard deviation, COV: Coefficient of variation, S.E.: Standard error, *Average of six estimation of tablet formulation, # Average of three estimation at each level of recovery

Table 3: Validation Parameters

Method	Drug	LOD* µg/ml	LOQ* µg/ml	Precision (% COV) Intraday n=6	Interday*		
					First day	Second day	Third day
I	ATV	0.2137	0.3318	0.8471	0.8974	0.7641	0.7654
	PIO	0.2143	0.5419	0.9084	0.9830	0.8741	0.9123

ATV: Atorvastatin Calcium, PIO: Pioglitazone**,** COV: Coefficient of variation, *Average of six determination

VALIDATION

Accuracy

To check the accuracy of the proposed methods, recovery studies were carried out at 80,100, and 120% of the test concentration as per ICH guidelines. The recovery study was performed three times at each level.The results of the recovery studies are given in Table 2.

Precision

Repeatability

To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Repeatability was performed for six times with tablets formulation. The standard deviation, coefficient of variation and standard error was calculated. The result of statistical evaluation are given in Table 2.

Intermediate Precision- (Inter-day and Intra-day precision)

The inter-day and intra-day precision was determined by assay of the sample solution on the same day and on different days at different time intervals respectively. The result of the same are presented in Table 3.

Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. For method-I the Beer- Lambert's concentration range was found to be 5-35 µg/ml for ATV and 5-40 µg/ml for PIO. The linearity data for both methods are presented in Table 1.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD & LOQ of ATV and PIO by proposed methods were determined using calibration standards. LOD and LOQ were calculated as $6.1\sigma/S$ and $13\sigma/S$, respectively, where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are shown in Table 3.

RESULT

Solubility studies indicated that aqueous solubility of ATV and PIO were enhanced more than 55 and 71 folds in 8.0 M sodium benzoate solution as compared to solubility in distilled water and buffer of pH 7.7 respectively. Linearity range for ATV and PIO were found to be 5-35 µg/ml and 5-40 µg/ml at respective selected wavelengths and coefficient of correlation were found 0.9998 for ATV at 313 nm and 0.9999, for PIO at 324 nm respectively (Table 1).The validity and reliability of proposed methods were assessed by recovery studies. Sample recovery for both the methods are in good agreement with their respective label claims, which suggested non interference of formulation additives and hydrotropic solubilizing agent sodium benzoate in estimation. (Table-2)Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval time and inter-assay precision. The standard deviation, coefficient of variance and standard error were calculated for ATV and PIO. The results were mentioned in Table 2. Intermediate precision study expresses within laboratory variation in different days. In both intra and inter-day precision study for both the methods %COV were not more than 2.0% indicates good repeatability and intermediate precision (Table 3).The value of LOD and LOQ were 0.2137µg/ml, 0.3318 µg/ml for ATV and 0.2143 µg/ml, 0.5419 for PIO in method I.

DISCUSSION

The present paper describes application of hydrotropic solubilization phenomenon for the simultaneous estimation of ATV and PIO in tablet dosage form by simultaneous equation method. Both drugs showed good regression values at their respective wavelengths and the results of recovery study reveled that any small change in the drug concentration in the solution could be accurately determined by the proposed methods and low values of LOD and LOQ indicated good sensitivity of proposed methods. Hence proposed methods are new, simple, cost effective, accurate, sensitive, and precise and can be adopted for routine analysis of ATV and PIO in tablet dosage form. Further, as sodium benzoate does not absorb above 332 nm, a large number of drugs having λmax above 332 nm can be used for estimation by proposed methods.

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