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Development and validation of Analytical methods for Simultaneous Estimation of Atorvastatin calcium and Irbesartan in Pharmaceutical dosage form

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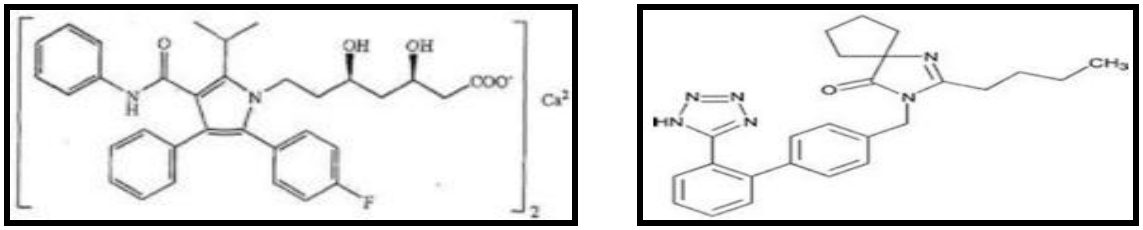
ABSTRACT

A simple, accurate and precise UV Spectrophotometric method (Simultaneous Equation Method) developed for the simultaneous estimation of Atorvastatin calcium and Irbesartan in pharmaceutical dosage form. The drugs were determined by Absorptivity values of Atorvastatin calcium and Irbesartan at selected wavelength 246nm and 228nm for ATOR and IRBE respectively. In this method drugs obey Beer's law using the concentration range 6-26µg/ml and 8-18µg/ml for ATOR and IRBE respectively. The correlation coefficient of ATOR and IRBE was found to be 0.992 and 0.996 respectively. The results of Recovery study for ATOR and IRBE were found to be within range of 98-102%. Precision study showed that %RSD was within range of acceptance limits (<2%). The method was validated as per ICH Q2 (R1) guideline.

Keywords: *Atorvastatin calcium (ATOR) and Irbesartan (IRBE), Simultaneous Equation method, UV Spectrophotometry.*

INTRODUCTION

Atorvastatin Calcium 7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxy heptanoate [Fig. 1a], a HMGCoA reductase inhibitor. It is for the treatment of dyslipidemia and the prevention of cardiovascular disease(heart attack, stroke, Myocardial infarction and stroke prophylaxis in patients with type II diabetes). ATOR is official in IP, BP and USP [1-3]. Irbesartan 2-butyl-3-({4-[2-(2H-1,2,3,4-tetrazol-5yl)phenyl]phenyl}methyl)-1,3-diazaspiro[4.4]non-1-en-4-one [Fig. 1b] is potent Adrenergic alpha-Agonists Both drugs are formulated together in the form of tablet dosage form for treatment of hypertension. The chemical structures of both drugs [4-7] were shown in figure 1. Irbesartan is a nonpeptide tetrazole derivative and an angiotensin II antagonist that selectively blocks the binding of angiotensin II to the AT1 receptor. It is used for the treatment of hypertension, as well as diabetic nephropathy with an elevated serum creatinine and proteinuria (>300 mg/day) in patients with type 2 diabetes and hypertension.



(a) **(b)**

Fig. 1: Chemical structure of (a) Atorvastatin Calcium and (b) Irbesartan

From literature survey it reveals that various analytical methods have been reported for estimation of Atorvastatin calcium and Irbesartan individually or in combination with other drugs either as API or in pharmaceutical dosage form. So the purpose of this work was to develop a simple, precise, accurate and sensitive Simultaneous Equation Method for determination of Atorvastatin calcium and Irbesartan in pharmaceutical dosage form.

MATERIALS AND METHODS

Instruments:

The instrument used was double beam UV- visible spectrophotometer (Shimadzu, model 1800, software: UV-Probe 2.34) having two matched quartz cell with 1 cm path length. Sonication of sample solutions was done using ultrasonic cleaner.

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Materials:

Atorvastatin Calcium (ATOR) drug sample was procured from Unicure Pharmaceuticals, Vadodara and Irbesartan (IRBE) drug sample was gifted by Alembic Pharma, Vadodara.

Method:

Preparation of standard stock solution:

The stock solution having 1000µg/ml concentration of ATOR and IRBE were prepared separately by dissolving accurately weighed 100mg of both drugs in 100 ml methanol. Further dilutions of standard stock solutions of both drugs were made with methanol to get the working standard stock solutions of 100µg/ml concentration of ATOR and 100µg/ml IRBE.

Method Development (Simultaneous Equation Method):

Selection of scanning range and sampling wavelength:

The standard stock solution of ATOR and IRBE were diluted with methanol individually to get the concentration of 10µg/ml for both and was scanned in UV range 200-400 nm. The λmax of both the drugs were found to be 246nm and 228nm respectively for ATOR and IRBE respectively in normal UV spectra shown in figure 2.

Development of Simultaneous Equation Method:

Preparation of Calibration curve:

Appropriate volume of aliquots from standard stock solutions of ATOR and IRBE were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with the methanol to obtain concentration of 6, 10, 14, 18, 22 and 26µg/ml for ATOR and 8, 10, 12, 14, 16, 18 µg/ml for IRBE respectively. The absorbance vs. concentration was plotted at 246 and 228nm. The straight-line equation was determined.

Simultaneous Equation Method:

The method was based on the absorption of drugs (X and Y) at the maximum wavelength. Other quantification analyses of ATOR and IRBE in Synthetic mixture were performed with the following equations [8, 9]:

CX = (A2ay1- A1ay2)/ ax2ay1-ax1ay…….. (1)
CY = (A1ax2 – A2ax1)/ ax2ay1-ax12ay……. (2)

Where CX and CY2 are the concentrations of X and Y drugs respectively in the diluted sample; ax1 and ax2 are absorptivity of X at λ1 and λ2; and ay1 and ay are absorptivity of Yat λ1 and λ2. The absorbance of the dilute samples at λ1 and λ2 are A1 (A1= ax1bCx+ ay1bcy) and A2 (A2= ax2bCx+ ay2bcy) respectively.

Method Validation:

The above proposed method was validated as per the ICH Q2 (R1) guidelines for validation of analytical procedures [10] in order to determine the linearity, Accuracy, Precision, LOD and LOQ.

Linearity and Range:

Calibration curve constructed was linear over a selected range of 6-16µg/ml for ATOR and 8-18 µg/ml for IRBE. The calibration curve of absorbance Vs concentration plotted was shown in figure 4 and 5. Each concentration was repeated six times. Correlation coefficient and regression line equations for ATOR and IRBE were calculated and were shown in table no.1.

Accuracy:

The accuracy of the developed method was determined by finding out the amount of recovery of Atorvastatin Calcium and Irbesartan. For the accuracy standard addition method was used where, as known amount of ATOR and IRBE were added to the known concentration (6µg/ml ATOR and 8µg/ml IRBE). The amount recovered was found by measuring the absorbance of the solution and was expressed as mean recovery of samples with upper and lower limits of percent relatives of standard deviation. Recovery was done at three different levels i.e. 80%, 100% and 120%, within the linearity range of both the drugs.

Precision:

Repeatability (n=6):

For the repeatability study, from the working stock solution, appropriate volume of solution was transferred to a 10 ml volumetric flask and diluted upto mark with methanol such that it gives the concentration of 10µg/ml and 10µg/ml of ATOR and IRBE respectively. The absorbance of the solutions was measured at 246nm and 228nm respectively. The procedure was repeated six times and % RSD was calculated and shown in table no. 3.

Intraday Precision (n=3):

From the working stock solution, appropriate volume of solution was transferred to a 10 ml volumetric flask and diluted up to mark with methanol such that it gives the concentration of 10, 14 and 18µg/ml of ATOR and 10, 12, and 14µg/ml of IRBE. The solutions were analysed three times on the same day and % RSD was calculated and shown in table no. 3.

Interday Precision (n=3):

From the working stock solution, appropriate volume of solution was transferred to a 10 ml volumetric flask and diluted up to mark with methanol such that it gives the concentration of 10, 14 and 18µg/ml of ATOR and 10, 12, and 14µg/ml of IRBE. The solutions were analysed three times on three different days and % RSD was calculated and were shown in table no. 4.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

Limit of detection (LOD) is the minimum concentration of the analyte in the sample which IRBE be analysed by the instrument. Limit of quantification (LOQ) is the minimum concentration of the analyte that IRBE be reliably quantified. The Limit of detection (LOD) and Limit of quantification (LOQ) were measured using following formula. The values of LOD and LOQ for ATOR and IRBE were shown in table no. 5.

LOD = 3.3 × (SD/Slope)
LOQ = 10 × (SD/Slope)

Where, SD = Standard deviation of the Y- intercepts of the 6 calibration curves.
Slope = Mean slope of the 6 calibration curves.

Estimation of Atorvastatin Calcium and Irbesartan in Pharmaceutical dosage form:

Combination containing both Atorvastatin Calcium and Irbesartan were used for the study. Combine solution equivalent to 150mg of Atorvastatin Calcium and 20mg of Irbesartan and transferred in to a 100 ml volumetric flask to bring both drugs in 75:10 ratio and stock solution of this was prepared in methanol, sonicated for 15 min, the volume was adjusted up to the mark with same solvent. This stock solution contains Atorvastatin Calcium 1500µg/ml and Irbesartan 20µg/ml. Then the appropriate dilution of 15µg/ml (ATOR) and 2µg/ml (IRBE) was made using methanol as solvent. All the determinations were carried out in triplicate. The absorbance of the prepared solutions was measured at 228nm and 246nm and then the concentration of both the drug was calculated using the equation of the straight line representing the calibration curves for Atorvastatin Calcium and Irbesartan. The amount of the drug found in Combined Dosage form calculated was shown in table no. 6.

RESULT

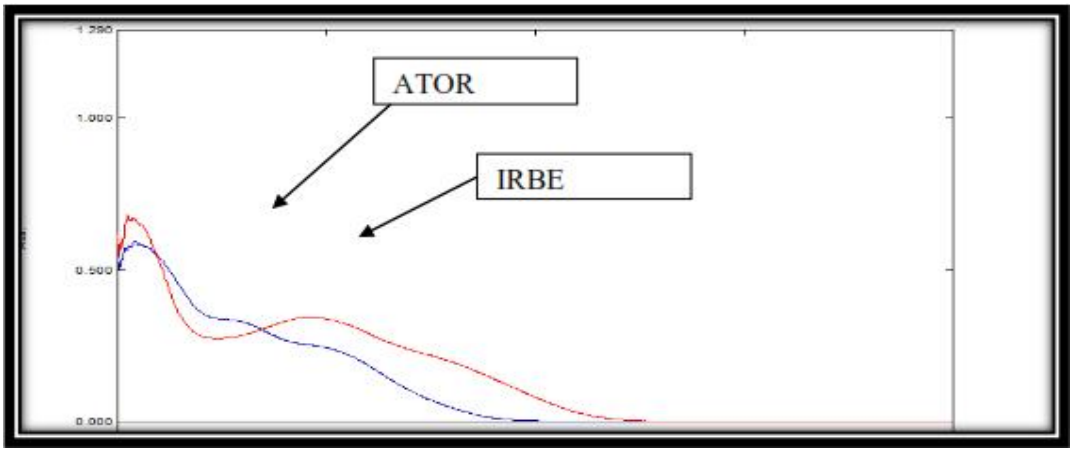

Fig. 2: Overlay spectra of ATOR(10µg/ml) and IRBE(10µg/ml) in methanol

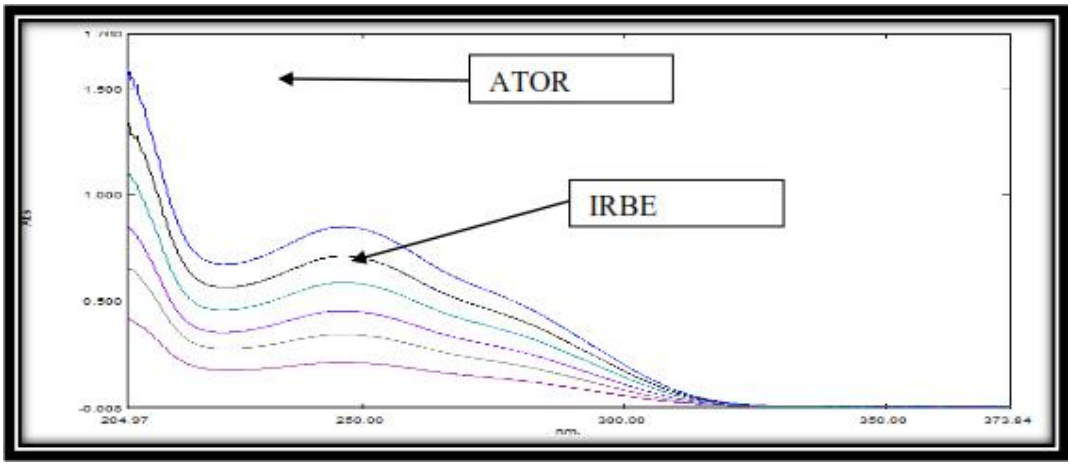

Fig. 3a: Overlain linearity spectra of ATOR in methanol.

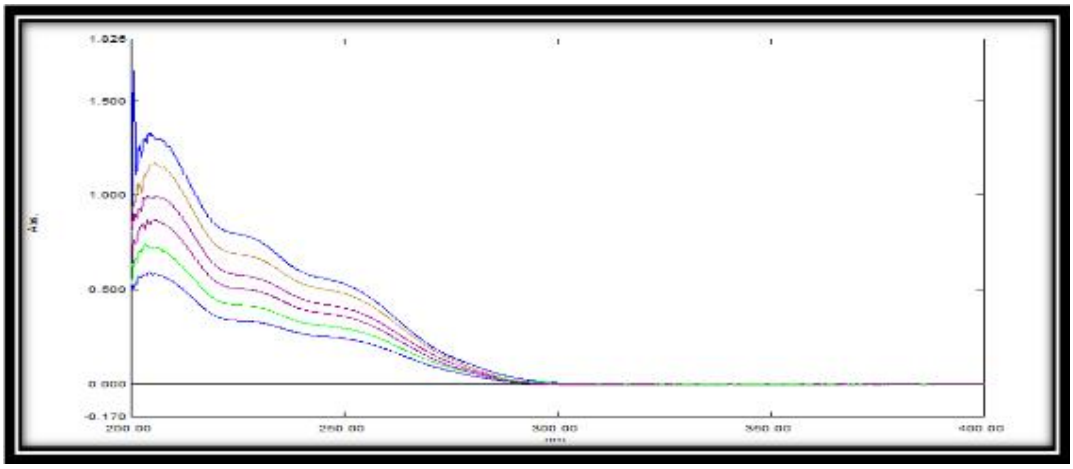

Fig. 3b: Overlain linearity spectra of IRBE in methanol.

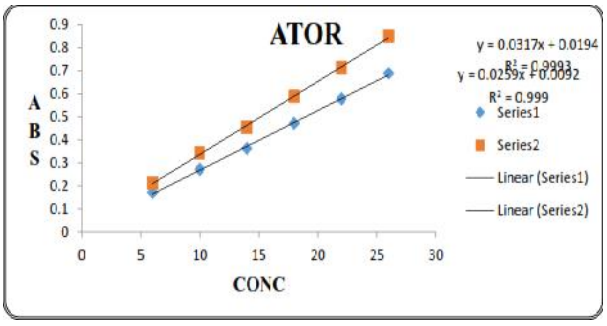

Fig. 4: Linearity graph of ATOR at 246nm and 228nm

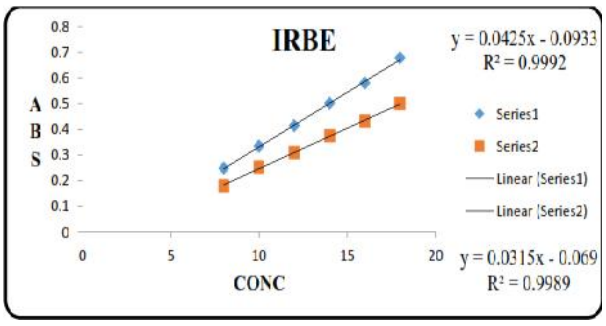

Fig. 5: Linearity graph of IRBE at 246nm and 228nm

Table No. 1: Optical Characteristics

Parameters	Atorvastatin Calcium	Irbesartan
Beer's law limit (µg/ml)	6-26µg/ml	8-18µg/ml
Regression equation	0.0259x + 0.0092	y = 0.0315x - 0.069
Correlation coefficient (R^2)	0.01583333	0.028
Standard deviation of Intercept (c)	0.000894	0.000983
Correlation coefficient (R^2)	0.999	0.998

Table No. 2: Results of Recovery studies

Drug	Concentration of STD drug	Recovery level (%)	Amount of drug added(µg/ml)	Amount of drug recovered(µg/ml)	% Mean recovery ±SD
ATOR	6	80	4.8	0.290	99.65%±1.021
		100	6	0.329	101.06%±1.470
		120	7.2	0.336	98.55%±1.69
IRBE	4	80	3.2	0.38	99.3%±1.25
		100	4	0.422	98.9%±0.88
		120	4.8	0.475	101.6%±1.74

Table No. 3: Repeatability, Inter-day and Intra-day precision of ATOR and IRBE

Drug	Concentration(µg/ml)	Average ABS±SD	%RSD
REPEATABILITY(n=6)			
ATOR	6	0.2715±0.0036	0.9476
IRBE	8	0.253±0.00314	1.2390
INTRADAY PRECISION(n=3)			
ATOR	10	0.2713± 0.00351	1.294
	14	0.451± 0.002	0.4434
	18	0.5866± 0.00565	0.9692
IRBE	10	0.254± 0.0025	0.9894
	12	0.309±0.0020	0.6729
	14	0.378±0.0035	0.9282
INTER DAY PRECISION(n=3)			
ATOR	10	0.279±0.0045	1.6140
	14	0.456±0.0030	0.694
	18	0.602±0.0010	1.6858
IRBE	10	0.261± 0.004	1.5325
	12	0.314± 0.0035	1.1172
	14	0.391± 0.0045	1.1720

***SD = standard deviation, ABS = Absorbance**

Table No. 4: Analysis of Pharmaceutical dosage form

Drugs	Label claim (mg/ml)	Conc. Taken for assay(µg/ml)	Absorbance of sample solution	Concentration found (µg/ml)	% Assay
ATOR	20	2	0.056	1.81	99.20%
IRBE	150	15	0.399	14.83	99.66%

Table No. 5: Limit of detection (LOD) and Limit of Quantification (LOQ)

Parameters	Atorvastatin Calcium	Irbesartan
LOD (µg/ml)	0.186417	0.115876
LOQ (µg/ml)	0.564901	0.35114

DISCUSSION

The present paper describes the estimation of ATOR and IRBE in combination by Simultaneous Equation method. The Beer-Lambert's concentration range was found to be 6-21µg/ml and 3.2-11.2µg/ml for both drug ATOR and IRBE at 235nm and 255nm respectively. The correlation coefficient was found to be 0.999 for ATOR and 0.998 for IRBE for proposed method. Precision was determined by studying repeatability, intraday and interday precision. The standard eviation and Relative standard deviation (%RSD) were calculated for both the drugs. The % RSD for proposed method were found to be not more than 2.0% which indicates good intermediate precision. The values of LOD and LOQ were 0.186417µg/ml and 0.564901µg/ml for ATOR and 0.115876 µg/ml and 0.35114µg/ml for IRBE respectively.

CONCLUSION

A simple, accurate and precise UV Spectrophotometric method (Simultaneous equation method) has been developed for the estimation of ATOR and IRBE in Synthetic mixture. It has advantage that it eliminates the spectral interference from one of the two drugs while estimating the other drug at selected wavelength.

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% Assay:

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