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RESEARCH ARTICLE

Development and Validation of Analytical Method for Simultaneous Estimation of Valsartan and Pioglitazone Hydrochloride by Simultaneous Equation Method

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ABSTRACT

The objective of the work is to develop analytical method for simultaneous estimation of Valsartan (VAL) and Pioglitazone hydrochloride (PIO). This method involve solving of simultaneous equations based on measurement of absorbances at two wavelengths 248 nm and 268 nm from 0.1N HCl solution and from phosphate buffer solution. Both the drugs obey the Beer's law in the concentration ranges employed for this method. Results of the methods were validated statistically. Novel, simple, sensitive, rapid, accurate and economical spectrophotometric methods have been developed for simultaneous estimation of Valsartan and Pioglitazone hydrochloride. The method can be used to estimate the amount of Valsartan and Pioglitazone hydrochloride in formulation containing Valsartan and Pioglitazone hydrochloride.

KEYWORDS

Valsartan, Pioglitazone hydrochloride, Anti hypertensive, Anti diabetic, Spectrophotometric analysis, Simultaneous equation method

INTRODUCTION

Valsartan, $[C_{24}H_{29}N_5O_3]$[1]is an angiotensin II receptor antagonist, which selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands.[1] It effectively inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure. Valsartan is selective for AT1 and has virtually no affinity for AT2. Inhibition of aldosterone secretion may inhibit sodium and water reabsorption in the kidneys while decreasing potassium excretion. It may be used as a first line agent to treat uncomplicated hypertension,[1] isolated systolic hypertension and left ventricular hypertrophy. May be used as a first line agent to delay progression of diabetes, isolated systolic hypertension and left ventricular hypertrophy and to delay progression of diabetic nephrotrophy.[1]

Pioglitazone hydrochloride $[C_{19}H_{20}N_2O_3]$ is an agonist at peroxisome proliferator activated receptors (PPAR) in target tissues for insulin action such as adipose tissue, skeletal muscle and liver.[2] It is used in diabetes mellitus. Activation of PPAR-gamma receptors increases the transcription of insulin-responsive genes involved in the control of glucose production, transport and utilization. In this way, Pioglitazone enhances tissue sensitivity to insulin and also reduces hepatic gluconeogenesis.[2] Thus, insulin resistance associated with type 2 diabetes mellitus is

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improved without an increase in insulin secretion by pancreatic β cells.[2]

Literature survey reveals that few HPLC and LC MS methods have been reported for determination of antihypertensive as single component in bulk, in formulations.[3,4,5,6,7,8,9,10] Also, not a single UV method is reported for simultaneous analysis of Valsartan and Pioglitazone hydrochloride. A successful attempt has been made to estimate two drugs simultaneously by spectrophotometric analysis. The objective of the investigation is to develop and validate an analytical method for the estimation of Valsartan and Pioglitazone HCl in a combined mixture by simultaneous UV spectroscopic method.

MATERIALS AND METHODS

A double beam UV/Visible spectrophotometer (Labtronic-LT2900) was employed with spectral bandwidth of 1 nm and wavelength accuracy of ± 0.3 nm with automatic wavelength correction with a pair of 10 mm quartz cells. A Shimadzu electronic analytical balance (BL – 220H) was used for weighing the sample. Valsartan (Cadila Pharmaceuticals, Ahmadabad), Pioglitazone hydrochloride (Aarti Drugs, Mumbai), HCl and phosphate buffer were used in the study.

Preparation of Calibration Curve

Preparation of Standard Calibration Curve of Valsartan in 0.1 N HCl Solution

Accurately weighed 50 mg of Valsartan was dissolved in 500 ml of 0.1 N HCl solution (stock solution).Then 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 ml of above solution was transferred in a 100 ml volumetric flask and volume was made up to the mark with 0.1 N HCl solution to make 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 µg/ml concentration.

The absorbance of each of these solutions were measured at the selected wavelengths (i.e.248 nm and 268 nm) using UV spectrophotometer and plotted against concentration. The concentration range over which the drugs obeyed beer's law was chosen.

Preparation of Standard Calibration Curve of Valsartan in pH 6.8 Phosphate Buffer Solution

Accurately weighed 50 mg of Valsartan was dissolved in 500 ml of pH 6.8 phosphate buffer solution (stock solution). Then 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45and 50 ml of above solution was transferred in a 100 ml volumetric flask and volume was made up to the mark with pH 6.8 phosphate buffer solution to make 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45and 50 µg/ml concentration.

The absorbance of each of these solutions were measured at the selected wavelengths (i.e., 248nm and 268 nm) using UV spectrophotometer. The calibration curve was plotted for 1to 50 µg/ml concentration at 248 nm and 268 nm.

Preparation of Standard Calibration Curve of Pioglitazone HCl in 0.1 N HCl Solution

Accurately weighed 50 mg of Pioglitazone HCl was dissolved in 500 ml of 0.1 N HCl Solution (Stock solution).Then 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 ml of above solution was transferred in a 100 ml volumetric flask and volume was made up to the mark with 0.1 N HCl solution to make 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 µg/ml concentration. The absorbance of each of these solutions were measured at the selected wavelengths (i.e., 248 nm and 268 nm) using UV spectrophotometer and plotted against concentration.

The concentration range over which the drugs obeyed beer's law was chosen. The range was found to be 1.0 to 50.0 µg/ml at both the wavelength.

Preparation of Standard Calibration Curve of Pioglitazone HCl in pH 6.8 Phosphate Buffer Solution

Accurately weighed 100 mg of Pioglitazone HCl was dissolved in 200 ml of pH 6.8phosphate buffer Solution (stock solution). Then, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0,10, 20, 30, 40 and 50 ml of above solution was transferred in a 50 ml volumetric flask and volume was made up to the mark with pH 6.8

phosphate buffer solution to make 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 100, 200, 300, 400 and 500 µg/ml concentration. The absorbance of each of these solutions were measured at the selected wavelengths (i.e., 248 nm and 268 nm) using UV spectrophotometer and plotted against concentration.

Development of Simultaneous Equation for Valsartan and Pioglitazone HCl

For 0.1 N HCl Solution

Absorptivity from all the concentration was calculated for both the drugs in 0.1 N HCl Solution and used for the development of simultaneous equation.[11,12]

$$Cx = \frac{(A2*ay1 - A1*ay2)}{ax2*ay1 - ax1*ay2} \quad (1)$$

$$Cy = \frac{(A1*ax2 - A2*ax1)}{(ax2*ay1 - ax1*ay2)} \quad (2)$$

The concentration of C_{VAL} and C_{PIO} can be obtained by solving equation (3) and (4).

$$CVAL = \frac{A1 \times 41.43 - A2 \times 20.33}{41.43 \times 21.5 - 20.33 \times 10.75} \quad (3)$$

$$CPIO = \frac{A2 \times 21.5 - A1 \times 21.5}{41.43 \times 21.5 - 20.33 \times 10.75} \quad (4)$$

Where,

1. 21.5 and 10.75 are absorptivity of Valsartan at λ_1 (248) and λ_2 (268) respectively.
2. 20.33and 41.43 are absorptivity of Pioglitazone HCl at λ_1 (248) and λ_2 (268) respectively.
3. A_1 and A_2 are absorbance of mixture at λ_1 (248) and λ_2 (268) respectively.
4. C_{VAL} and C_{PIO} are concentration in gm/liter.

For pH 6.8 Phosphate Buffer Solution

Absorptivity from all the concentration was calculated for both the drugs in pH 6.8 phosphate buffer solution and used for the development of simultaneous equation.

The concentration of C_{VAL} and C_{PIO} can be obtained by solving equation (5) and (6).

$$C_{VAL} = \frac{A1 \times 5.32 - A2 \times 5.9}{5.32 \times 31 - 5.9 \times 7.25} \quad (5)$$

$$C_{PIO} = \frac{A2 \times 31 - A1 \times 7.25}{5.32 \times 31 - 5.9 \times 7.25} \quad (6)$$

1. 31 and 7.25 are Absorptivity of Valsartan at λ_1 (248) and λ_2 (268) respectively.
2. 5.9 and 5.32 are Absorptivity of Pioglitazone HCl at λ_1 (248) and λ_2 (268) respectively.
3. A_1 and A_2 are absorbance of Mixture at λ_1 (248) and λ_2 (268) respectively.
4. C_{VAL} and C_{PIO} are Concentration in gm/liter

Standardization of the Method by Analysis of Powder Mixture of Known Composition

The mixture of Valsartan and Pioglitazone HCl having concentration of 40.0 µg/ml of VAL and 30.0 µg/ml of PIO were analyzed by preparing a solution of suitable dilution in 0.1N HCl and phosphate buffer pH 6.8. The absorbance of the solution at 248 nm and 268 nm for 0.11N HCl and at 248 nm and 268 nm for phosphate buffer pH 6.8 were measured. The values were substituted in equation (3) and (4) to get a concentration of Valsartan and Pioglitazone HCl respectively in 0.1N HCl solution. A concentration of Valsartan and Pioglitazone HCl in phosphate buffer pH 6.8 solution can be determined by substituting the absorbance values in equation (5) and (6).

The results of the analysis of powder mixture are reported in Table 10 and data for statistical validation is given in Table 11.

Procedure for Precision

In intraday precision sample having concentration of 40.0 µg/ml of VAL and 30.0 µg/ml of PIO was scanned six times at different time interval in the same day. Interday precision was obtained by the assay of six sample sets on different days. The results are shown in Table 12 and 13.

RESULTS AND DISCUSSION

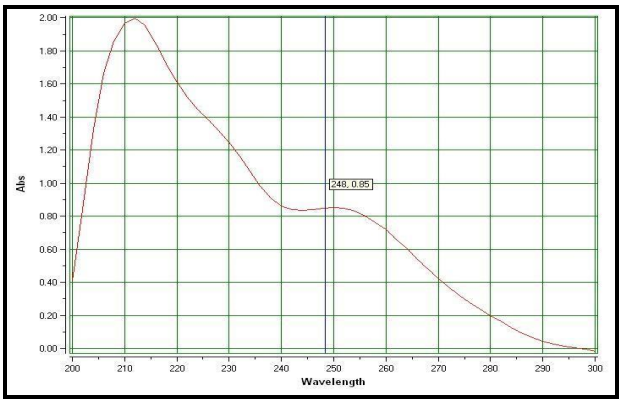

Figure 1: UV Spectrum of Valsartan in 0.1 N HCl Solution

Valsartan showed λ_{max} at 248nm in 0.1 N HCl solution.

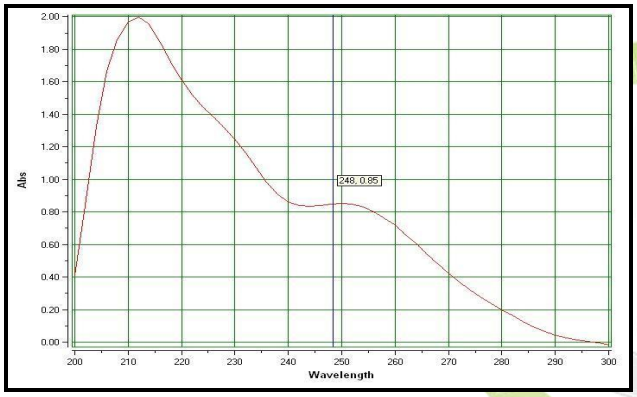

Figure 2: UV Spectrum of Valsartan in pH 6.8 Phosphate Buffer Solution

Valsartan showed λ_{max} at 248 nm in pH 6.8 Phosphate buffer solution.

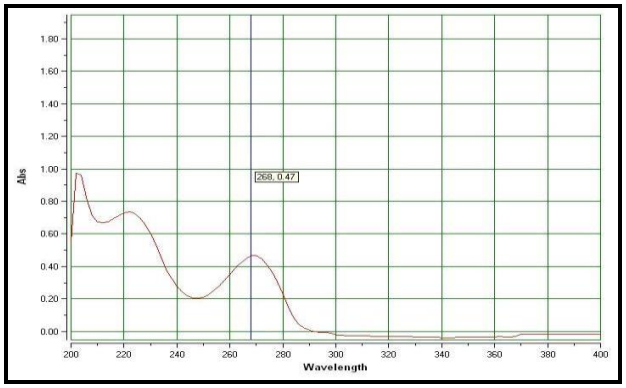

Figure 3: UV Spectrum of Pioglitazone HCl in 0.1 N HCl Solution

Pioglitazone HCl showed λ_{max} at 268 nm in 0.1 N HCl solution.

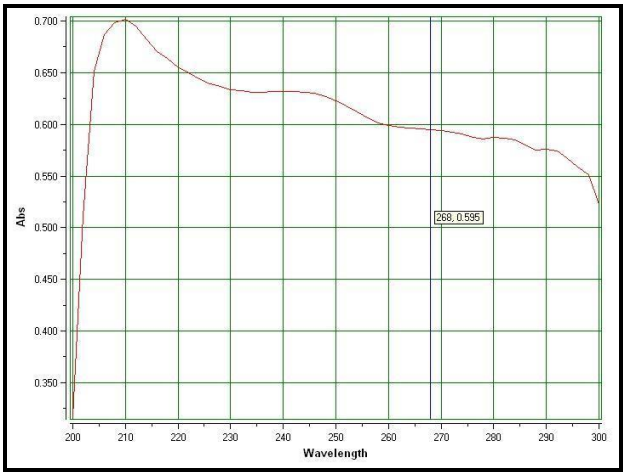

Figure 4: UV Spectrum of Pioglitazone HCl in pH 6.8 Phosphate Buffer Solution

Pioglitazone HCl showed λ_{max} at 268 nm in pH 6.8 Phosphate buffer Solution.

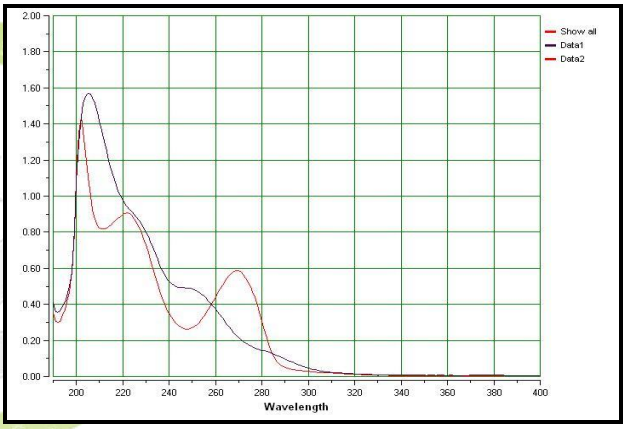

Figure 5: UV Overlay Spectrum of Valsartan and Pioglitazone HCl in 0.1 N HCl Solution

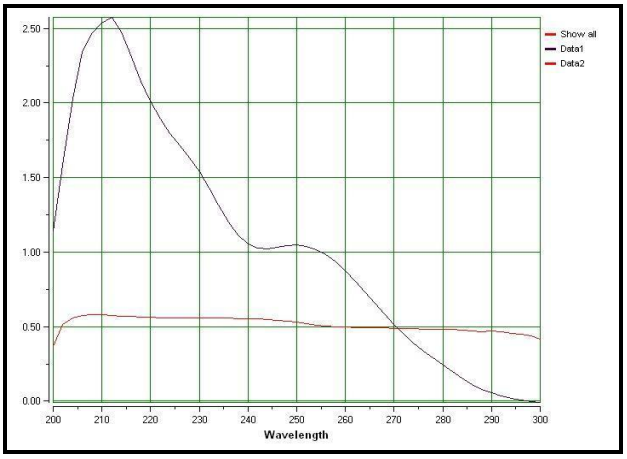

Figure 6: UV Overlay Spectrum of Valsartan and Pioglitazone HCl in pH 6.8Phosphate Buffer Solution

Table 1: Data for Standard Calibration Curve of Valsartan at 248 nm in 0.1 N HCl Solution

Sr. No.	Concentration (µg /ml)	Absorbance
1	1	0.025
2	2	0.049
3	5	0.112
4	10	0.197
5	15	0.296
6	20	0.403
7	25	0.511
8	30	0.626
9	35	0.744
10	40	0.841
11	45	0.966
12	50	1.043

*= mean absorbance of 3 absorbances

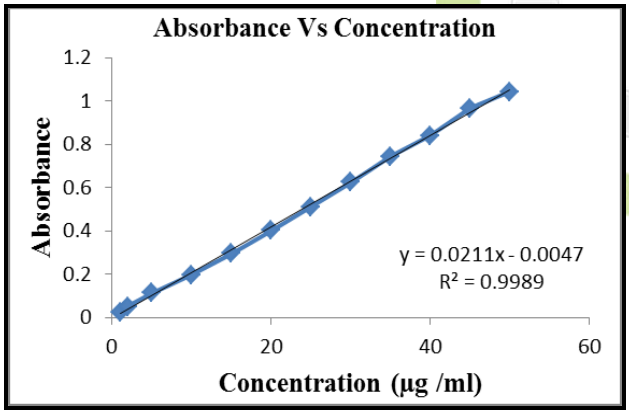

Figure 7: Calibration Curve of Valsartan at 248 nm in 0.1 N HCl Solution

Within the range of 1.0 to 50.0 µg/ml the drug obeyed Beer's law.

Table 2: Data for Standard Calibration Curve of Valsartan at 268 nm in 0.1 N HCl Solution

Sr. No.	Concentration (µg /ml)	Absorbance
1	1	0.018
2	2	0.024
3	5	0.059
4	10	0.082
5	15	0.180
6	20	0.208
7	25	0.239
8	30	0.285
9	35	0.332
10	40	0.360
11	45	0.397
12	50	0.458

*= mean absorbance of 3 absorbances

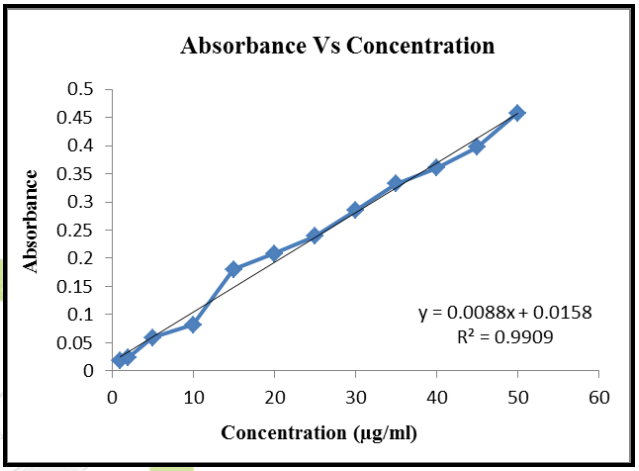

Figure 8: Calibration Curve of Valsartan at 268 nm in 0.1 N HCl Solution

Within the range of 1.0 to 50.0 µg/ml the drug obeyed Beer's law.

Table 3: Data for Standard Calibration Curve of Valsartan at 248 nm in pH 6.8 Buffer Solution

Sr. No.	Concentration (µg /ml)	Absorbance
1	1	0.166
2	2	0.273
3	5	0.468
4	10	0.634
5	15	0.808
6	20	0.984
7	25	1.14
8	30	1.197
9	35	1.344
10	40	1.516
11	50	1.932

*= mean absorbance of 3 absorbances

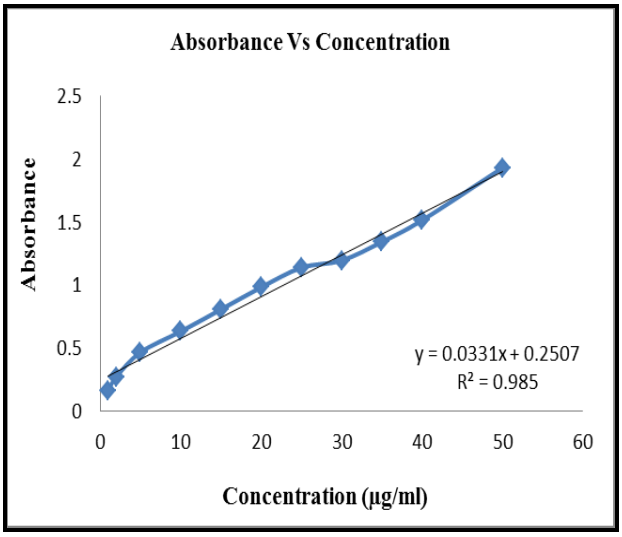

Figure 9: Calibration Curve of Valsartan at 248 nm in pH 6.8 Buffer Solution

Within the range of 1.0 to 50.0 µg/ml the drug obeyed Beer's law.

Table 4: Data for Standard Calibration Curve of Valsartan at 268 nm in pH 6.8 Buffer Solution

Sr. No.	Concentration (µg /ml)	Absorbance
1	1	0.042
2	2	0.086
3	5	0.166
4	10	0.268
5	15	0.383
6	20	0.472
7	25	0.548
8	30	0.596
9	35	0.662
10	40	0.736
11	45	0.824
12	50	0.953

*= mean absorbance of 3 absorbances

Table 5: Data for Standard Calibration Curve of Pioglitazone HCl at 268 nm in 0.1 N HCl Solution

Sr. No.	Concentration (µg /ml)	Absorbance
1	1	0.046
2	2	0.090
3	5	0.211
4	10	0.406
5	15	0.597
6	20	0.834
7	25	0.986
8	30	1.208
9	35	1.376
10	40	1.645
11	45	1.858
12	50	1.992

*= mean absorbance of 3 absorbances

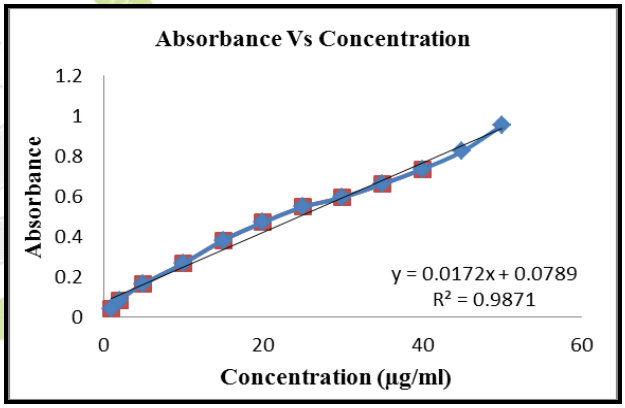

Figure 10: Calibration Curve of Valsartan at 268 nm in pH 6.8 Buffer Solution

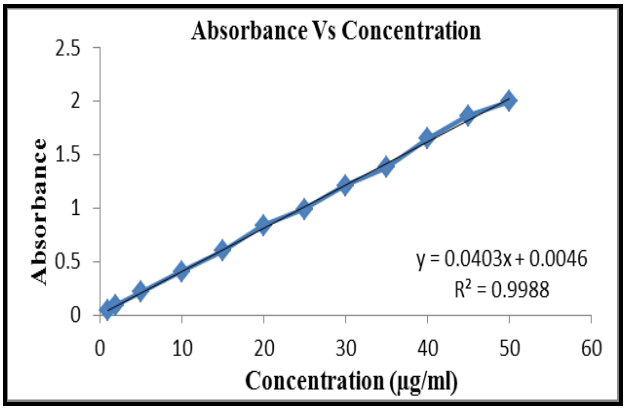

Figure 11: Calibration Curve of Pioglitazone HCl at 268 nm in 0.1 N HCl Solution

Here, within the range of 1.0 to 50.0 µg/ml the drug obeyed Beer's law.

Table 6: Standard Calibration Curve of Pioglitazone HCl at 248 nm in 0.1 N HCl Solution

Sr. No.	Concentration (µg /ml)	Absorbance
1	1	0.022
2	2	0.040
3	5	0.100
4	10	0.200
5	15	0.307
6	20	0.413
7	25	0.451
8	30	0.608
9	35	0.716
10	40	0.826
11	45	0.917
12	50	1.030

*= mean absorbance of 3 absorbance

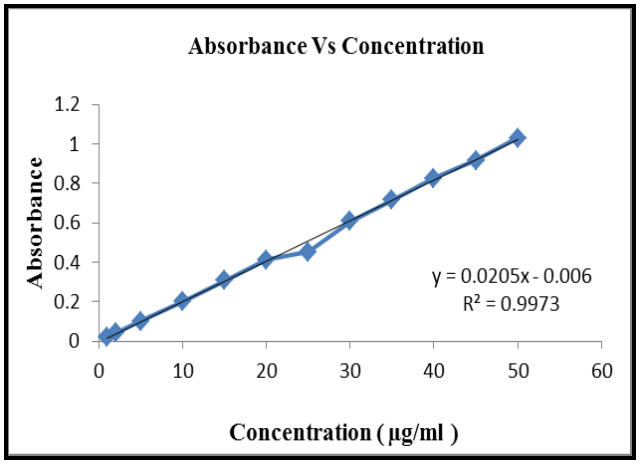

Figure 12: Linearity Relationship between Absorbance Vs Concentration

Within the range of 1.0 to 50.0 µg/ml the drug obeyed Beer's law.

Table 7: Data for Standard Calibration Curve of Pioglitazone HCl at 248 nm in pH 6.8 Buffer Solution

Sr. No.	Concentration (µg /ml)	Absorbance
1	5	0.035
2	10	0.082
3	20	0.121
4	30	0.191
5	40	0.276
6	50	0.360
7	100	0.575
8	200	0.954
9	300	1.294
10	400	1.706
11	500	2.076

*= mean absorbance of 3 absorbance

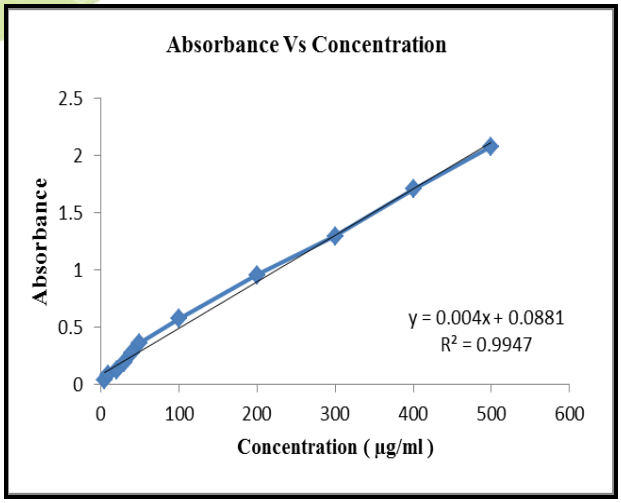

Figure 13: Calibration Curve of Pioglitazone HCl at 248 nm in pH 6.8 Buffer Solution

Within the range of 5.0 to 500.0 µg/ml the drug obeyed Beer's law.

Table 8: Data for Standard Calibration Curve of Pioglitazone HCl at 268 nm in pH 6.8 Buffer Solution

Sr. No.	Concentration (µg /ml)	Absorbance
1	5	0.017
2	10	0.040
3	15	0.088
4	20	0.132
5	25	0.182
6	30	0.207
7	35	0.233
8	40	0.244
9	45	0.288
10	50	0.308
11	100	0.562
12	200	0.849
13	300	1.076
14	400	1.322
15	500	1.715

*= mean absorbance of 3 absorbances

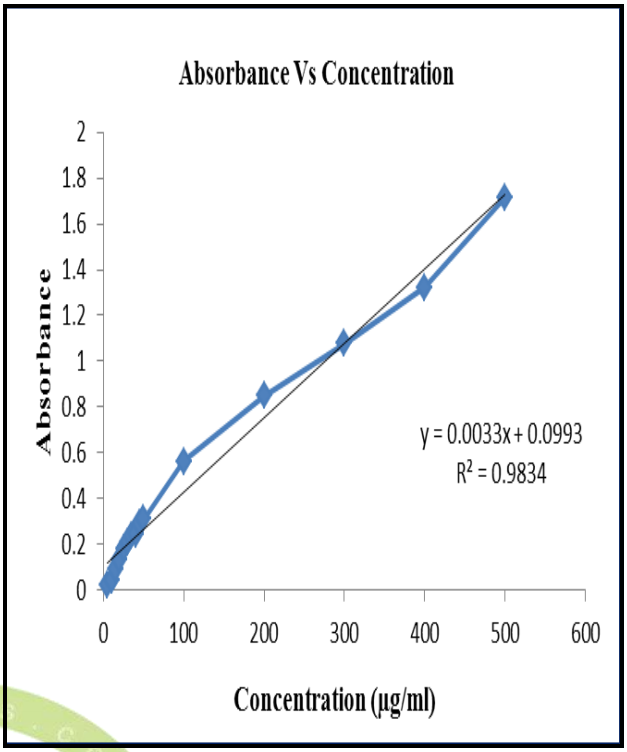

Figure 14: Calibration Curve of Pioglitazone HCl at 268 nm in pH 6.8 Buffer Solution

Within the range of 5.0 to 500.0 µg/ml the drug obeyed Beer's law.

Table 9: Regression and Optical Characteristics of Valsartan and Pioglitazone HCl

Parameters	Valsartan		Pioglitazone HCl	
	In 0.1N HCl	In Phosphate buffer pH6.8	In 0.1N HCl	In Phosphate buffer pH6.8
Working λ	248	248	268	268
Beer's Law range	1 – 50 µg/ml	1 - 50 µg/ml	1 - 50 µg/ml	5 - 500 µg/ml
Molar absorptivity (l / mole.cm)	82155.67	176417.2	158983.2	16076.80
Regression Values:				
i. Slope	0.021	0.033	0.040	0.003
ii. Intercept	-0.004	0.250	0.004	0.099
iii. Regression coefficient (r^2)	0.998	0.985	0.998	0.983

Table 10: Data for Powder Mixture Analysis

Sr. No.	Amount present in µg/ml		Amount found in µg/ml		Amount found in %	
	VAL	PIO	VAL	PIO	VAL	PIO
1.	30	30	29.2	29.61	97.33	98.70
2.	30	30	29.56	29.98	98.53	99.93
3.	30	30	29.36	30.65	97.87	102.17
4.	30	30	29.59	30.29	98.63	100.97
5.	30	30	30.15	29.96	100.50	99.87
6.	30	30	29.38	30.51	97.93	101.70

Table 11: Statistical Validation of Pure Drugs

Name of Component	Amount Present (µg/ml)	Mean*	Standard Deviation	% Co-efficient of Variation	Standard Error of Mean
Valsartan	30	98.47	1.105	1.122	0.4510
Pioglitazone HCl	30	100.56	1.296	1.289	0.5289

* Here Mean is the average of (n=6) results.

Table 12: Intra – Day Precision

Drug	% Mean*	S.D*	% R.S.D.*	S.E.*
VAL	99.38	1.306	1.314	0.5330
PIO	100.195	1.184	1.182	0.4832

* n=6

Table 13: Inter – Day Precision

Drug	% Mean*	S.D.*	% R.S.D.*	S.E.*
VAL	99.85	1.769	1.772	0.7222
PIO	99.51	1.349	1.356	0.5509

* n=6

DISCUSSION

Proposed method for simultaneous estimation of Valsartan and Pioglitazone HCl in combined sample solutions was found to be simple, accurate and reproducible. Table.10 shows data for optical characteristics. Data for validation and precision studies are given in Table 11, 12 and 13. Once the equations are determined, analysis required only the measuring of the absorbances of the sample solution at the two wavelengths selected, followed by a few simple calculations.

The standard deviation (S.D.), relative standard deviation (%R.S.D.) and standard error (S.E.) calculated are low, indicating high degree of precision of the method. The %R.S.D. is less than 2% as required by USP and ICH guidelines.

CONCLUSION

The method was successfully used to estimate the amount of Valsartan and Pioglitazone hydrochloride in marketed tablet formulation containing 5 mg of Valsartan and 12.5 mg of Pioglitazone hydrochloride. The results obtained were comparable with the corresponding labeled amounts, indicating non-interference of excipients in the estimation.

By observing validation parameters, method was found to be specific, accurate, precise, repeatable and reproducible. This method is simple in calculation, hence can be employed for routine analysis of tablet for assay as well as dissolution testing.

ACKNOWLEDGEMENT

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