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Deniz Çıkla Yılmaz
Analytical Chemistry Department, Faculty of Pharmacy, Marmara University, Istanbul, Turkey

Bilge Bebek
Faculty of Pharmacy, Marmara University, Istanbul, Turkey

Serap Karaderi
Analytical Chemistry Department, Faculty of Pharmacy, Marmara University, Istanbul, Turkey

Correspondence
Deniz Çıkla Yılmaz
Analytical Chemistry Department, Faculty of Pharmacy, Marmara University, Istanbul, Turkey

Forced degradation of valsartan: Development and validation of stability indicating RP-HPLC method

Deniz Çıkla Yılmaz, Bilge Bebek and Serap Karaderi

Abstract
In the present work, a new validated stability indicating HPLC method for quantitative determination of valsartan in tablet formulation was developed. The column was Inertsil ODS-3 (4.0 x 125 mm id; 5µm particle size) and the mobile phase was composed of 50 mM NaH_2PO_4 (pH 2,6) - methanol (35:65, v/v) with a flow rate 1 ml/min. Eluents were monitored by DAD detector at 254 nm. Calibration curve was linear in the concentration range 2,4 – 60 µg/ml (R^2>0.999). Valsartan was subjected to stress conditions including acidic, alkaline, oxidation, photolysis and thermal degradation. The developed method was found to give good separation between pure drug and degraded product. The proposed method was successfully applied for the stability assay of valsartan in tablet formulation and validated as per ICH guidelines.

Keywords: Valsartan, stability indicating HPLC method, forced degradation study, stress degradation

Introduction
Valsartan is chemically designated as N-(1-Oxopentyl)-N-[[2'-(1H-tetrazol-5-yl) [1,1'-biphenyl]-4-yl]methyl-L-valine. It is an antihypertensive drug which selectively inhibits angiotensin receptor type II [1]. The recent literature reveals the development of a spectrophotometric method which depends on complexation of valsartan with calcium and magnesium analysis from tablet form [2], voltammetric determination of Nickel complexation and analysis of valsartan in pharmaceuticals and urine [3], dissolution study to assess the quality of some commercially available valsartan tablets in Bangladesh market [4], a spectrofluorometric method for determination of valsartan in commercial tablets [5] and stability indicating HPLC methods for valsartan [6–8].
The present study illustrates development and validation of a simple, accurate and precise procedure for development and validation of a RP-HPLC method for the simultaneous estimation of valsartan in pharmaceutical dosage form

Materials and Methods
Chemicals and Reagents
Valsartan was purchased from Supelco. HPLC grade methanol and analytical grade NaH_2PO_4, 35% H_2O_2, HCl, NaOH were purchased from Merck. The mobile phase composition used in HPLC was 0.1 M NaH_2PO_4, pH adjusted to 2.6 using 0.1 M H_3PO_4, diluted and mixed with methanol in 35:65 (v/v) ratio. Then the mobile phase was filtered through a Nylon 0.45 µm membrane filter and degassed before use. Diovan® tablets were manufactured by Novartis, Turkey. They were labeled to contain 80 mg valsartan/tablet and were purchased from local market.

Instrument and Chromatographic Conditions
Chromatographic analysis was performed using the Agilent 1260 Infinity HPLC system equipped with Chem Station software, G1311B 1260 Quaternary Pump, G1315D 1260 DAD VL detector, G1316A 1260 Thermostated Column Compartment, G1329B 1200 Automatic Liquid Sampler. The detection was set at 254 nm and separation was carried out at temperature maintained at 22 °C using Inertsil ODS-3 (4.0 x 125 mm 5µm) column by isocratic elution with a flow rate of 1.0 ml/min. The injection volume was 10 µl. Mobile phase was used as diluent during the standard and test samples preparation. The other instruments used were pH meter, digital balance, ultrasonic bath, hot air oven.

Preparation of standard and sample solutions

Standard Solution Preparation

For the preparation of the standard valsartan stock solution, 30 mg of valsartan was accurately weighed and dissolved in methanol in a 100 ml volumetric flask. Aliquots of the standard stock solution of valsartan were transferred into 25 ml volumetric flasks and the solutions were made up to volume with mobile phase to give final concentration.

Tablet Sample Solution Preparation

Ten Diovan® tablet were weighted and crushed to obtain a fine powder. Powder equivalent to 80 mg of valsartan was weighted and transferred into 50 ml volumetric flask, dissolved and 50 ml methanol was added. The solution was sonicated in an ultrasonic bath for 30 minutes and the solution was filtered. The appropriate volume of the filtrate was diluted with the mobile phase to obtain 36 µg/ml standard solution for HPLC.

Forced Degradation Studies

Forced degradation studies were carried out under different conditions to determine whether the analytical method and assay were stability-indicating. Valsartan at a concentration of 0.05 mg/ml was used in all degradation studies. After completion of the degradation processes, the solutions were neutralized and diluted with mobile phase.

Hydrolytic degradation under acidic condition

5 ml of the stock solution was treated with 5 ml of 1 M HCl and kept on a water bath at 60 °C for 6 hours. Then 5 ml of 1 M NaOH was added for neutralization and diluted with mobile phase.

Hydrolytic degradation under alkaline condition

5 ml of the stock solution was treated with 5 ml of 1 M NaOH and kept on a water bath at 60 °C for 6 hours. Then 5 ml of 1 M HCl was added for neutralization and diluted with mobile phase.

Oxidative degradation

5 ml of the stock solution was treated with 5 ml of 7% H_2O_2 solution and kept on water bath at 60 °C for 6 hours and transferred to a volumetric flask, diluted with mobile phase.

Thermal induced degradation

Valsartan active pharmaceutical ingredient (API) powder was taken in petri dish and kept in Hot air oven at 60 °C for 6 hours. Then the sample was taken and diluted with mobile phase.

Photo Degradation

Valsartan active pharmaceutical ingredient (API) powder was taken in petri dish and kept in photo stability chamber at 254 nm for 8 hours and diluted with mobile phase.

Results and Discussion

Optimization of Chromatographic Conditions

Preliminary experiments were performed using Inertsil ODS-3 (4.0 x 125 mm 5µm) column. The pKa of Valsartan is 4,9 [1], therefore a buffer with acidic pH (NaH_2PO_4 at pH 2,6) was selected. The effect of the buffer salt concentration on separation was also investigated by changing its concentration from 0.025 M to 0.1 M. various ratios of methanol were used to get symmetrical valsartan peak and to separate all the valsartan degradation products. Flow rate was 1 ml/min, column oven temperature was fixed at 22°C. DAD detector was set at 210 and 254 nm. In this optimized method System suitability parameters were given in Table 1 and the representative chromatogram for valsartan in Fig 1.

Method Validation

The described method has been validated for specificity, linearity, limit of detection, limit of quantitation, accuracy, precision and robustness as per The International Conference on Harmonisation (ICH) guidelines [9].

Specificity

Forced degradation studies were performed to confirm the stability indicating properties and specificity of the method. Valsartan was subjected to ICH recommended forced degradation conditions. It is concluded that at room temperature valsartan is stable in acidic, basic, thermal, oxidative and photolytic stress conditions. At 60 °C basic and thermal degradation studies showed no additional peak but under oxidative and acidic stress valsartan degraded up to 19.77% and 23.61%, respectively. The developed method effectively separated the degradation products from valsartan peak Fig 3-7. Peak purity results were greater than 0.990 which means valsartan peak is homogeneous.

Linearity and Range

For linearity assessment, replicates (n = 3) of seven concentrations were analyzed in the range of 2.4 to 60 µg/ml. Calibration curve was constructed by plotting average peak area against concentration and then the regression equation was computed. Linear relationship was evaluated using the least square method within Microsoft Excel® program. Linear regression equation was y = 17.257x - 9.4717 with a correlation coefficient of R^2 = 0.9996. Linearity curve of valsartan is shown in Fig 8.

Limit of Detection and Limit of Quantitation

Limit of detection (LOD) and limit of quantitation (LOQ) were determined by signal to noise ratio of 3:1 and 10:1. LOD and LOQ of the method found to be 1.2 µg/ml and 2.4 µg/ml respectively.

Accuracy

Accuracy of the proposed method was determined by performing the recovery experiment. The recovery experiment was studied by adding known amount of standard valsartan to the pharmaceutical product and calculating the recovered standard amount. At 50%, 100% and 150% standard addition level mean recovery of valsartan found to be 100.76%, 99.28% and 100.41% respectively. The results of recovery experiment are given in Table 3.

Precision

Method precision was assessed by intraday (repeatability) and interday (intermediate precision) repeatability. The intraday precision was done by analyzing 60 µg/ml standard solution of valsartan for six injections on the same day. Interday precision study was performed at two different concentration levels (36 and 60 µg/ml) for 3 days. % RSD values were ≤2 indicates acceptable precision of the method and the results are shown in Table 5a and 5b.

Robustness

For assessment of robustness of the proposed method, various parameters including mobile phase ratio, buffer pH and temperature were chosen. Then the effect on the chromatographic parameters (retention time, plate count, tailing factor) was investigated. The analyzed conditions and obtained results are shown in Table 6.

Table 1: System suitability parameters

Property	Values	Required limits
Resolution (Rs)*	3.13555	Rs >2
Tailing factor (T)	1.00832	T <2
Capacity factor (k')	4.978	k' >2
Theoretical plate (N)	3171	plate N >2000

* between preceding degredation peak and valsartan

Table 2: Summary of forced degradation study

Stress conditions	Time (h)	% Degradation room temperature	% Degradation 60°C	Peak Purity
1 M HCl	6	Not degraded	23.61	999.931
1 M NaOH	6	Not degraded	Not degraded	999.927
7% H2O2	6	Not degraded	19.77	999.988
Thermal	6	Not degraded	Not degraded	999.989
Photo degradation	8	Not degraded	Not degraded	999.987

Table 3: Results of the accuracy study

Accuracy Level	Amount of drug taken(mg)	Amount of drug spiked (mg)	Recovery %	RSD% (n=3)
50	80	40	100.76	0,32
100	80	80	99.28	0,27
150	80	160	100.41	0,41

Table 4: Assay results for the tablet dosage form

Labeled claim (mg)	Amount found (mg)	SD	RSD% (n=3)
80	80.20	0.60	0.748

Table 5a: Intraday precision study

Sr. No.	Intraday precision (C = 60 µg/ml)	
	Retention time	Peak area
1	6.971	1004.10455
2	6.979	1003.14478
3	6.978	1002.62122
4	6.980	1007.30957
5	6.920	1005.40826
6	6.892	1007.30957
Average	6.953	1004.98299
SD	0.038	2.03644
%RSD	0.544	0.20263

Table 5b: Interday precision study

	Interday precision			
	(C = 36 µg/ml)(n=3)		(C = 60 µg/ml)(n=3)	
	Retention time	Peak area	Retention time	Peak area
Average	6,784	584,61908	6.964	1.033,12557
SD	0.015	1,08818	0.005	1,05229
RSD %	0.220	0,186	0.071	0,102

Table 6: Results of robustness study

Parameter		Retention time	Theoretical plates	Tailing factor
Buffer: Methanol (35:65± 2%)	(33:67)	5.571	3030	1.00957
	(35:65)	6.883	3049	1.01613
	(37:63)	8.757	3329	0.98319
Buffer pH (2.6 ± 0.1)	2.5	6.895	3232	1.01075
	2.6	6.886	3272	0.99474
	2.7	6.749	3259	0.98883
Temperature (°C) (22°C ± 2)	20	7.110	3006	1.01286
	22	6.888	3173	1.03908
	24	6.724	3224	1.01630

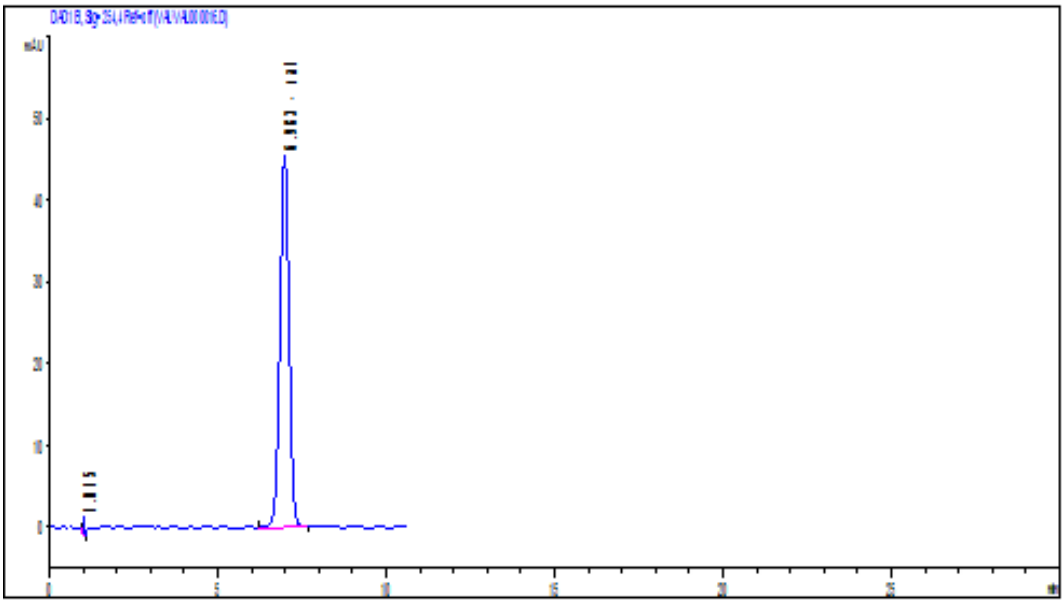

Fig 1: Valsartan Standard

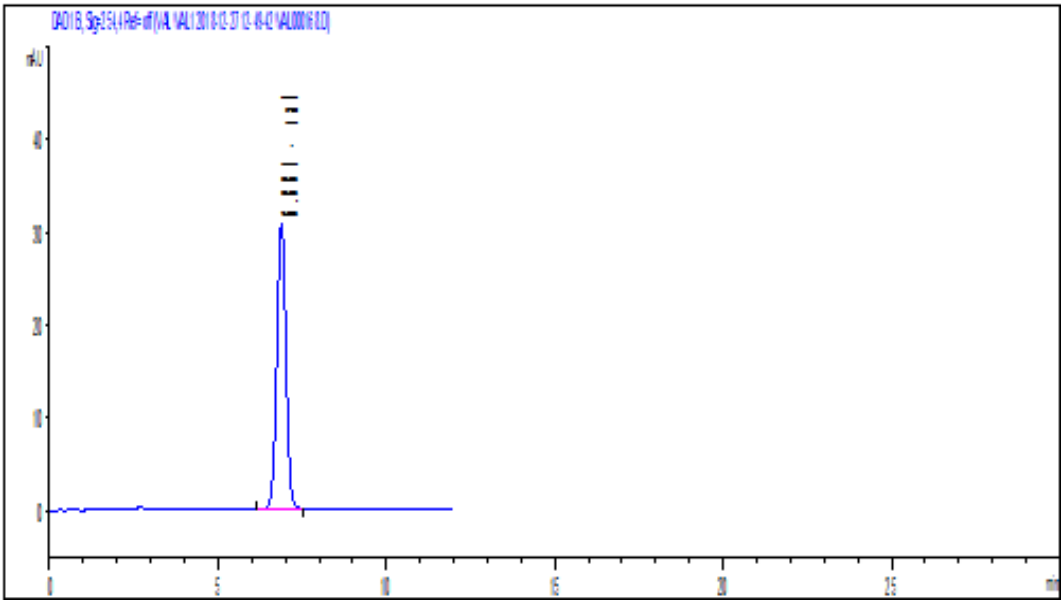

Fig 2: Chromatogram obtained from Diovan tablet formulation

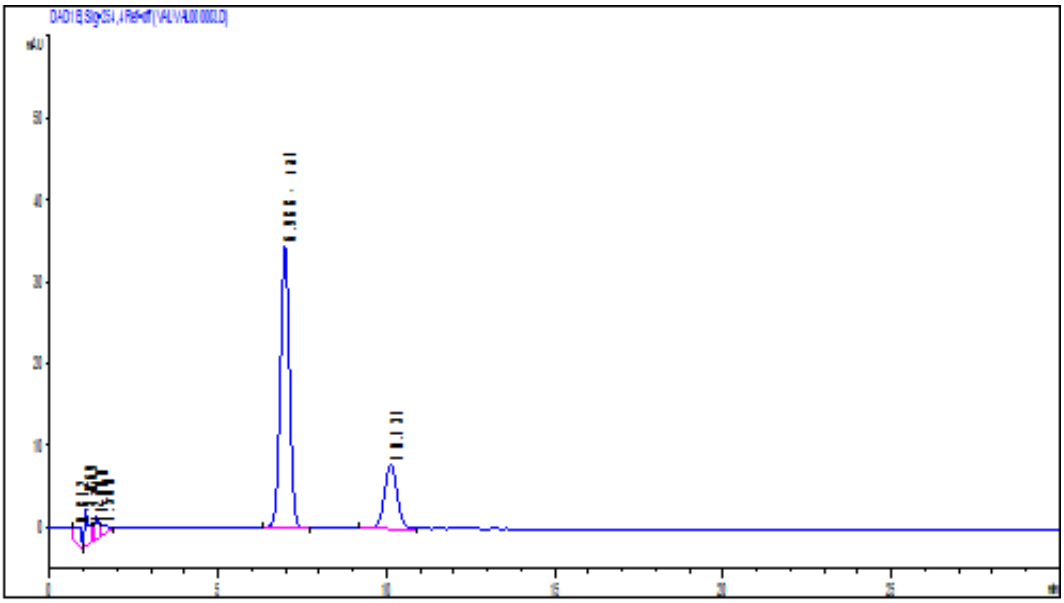

Fig 3: Valsartan after treatment with 1N HCl at 60 °C for 6 hours

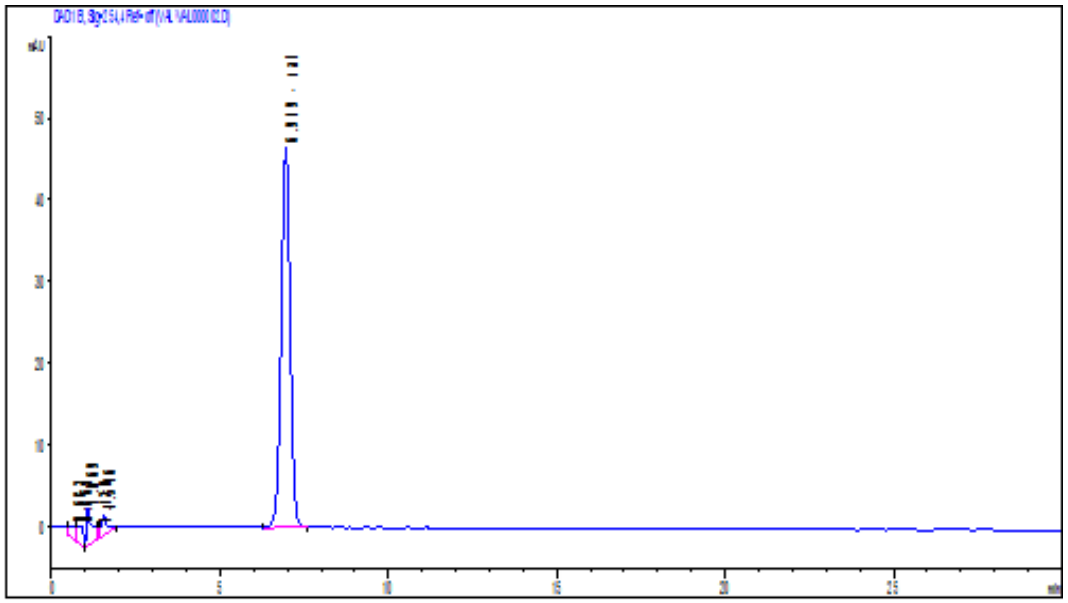

Fig 4: Valsartan after treatment with 1N NaOH at 60 °C for 6 hours

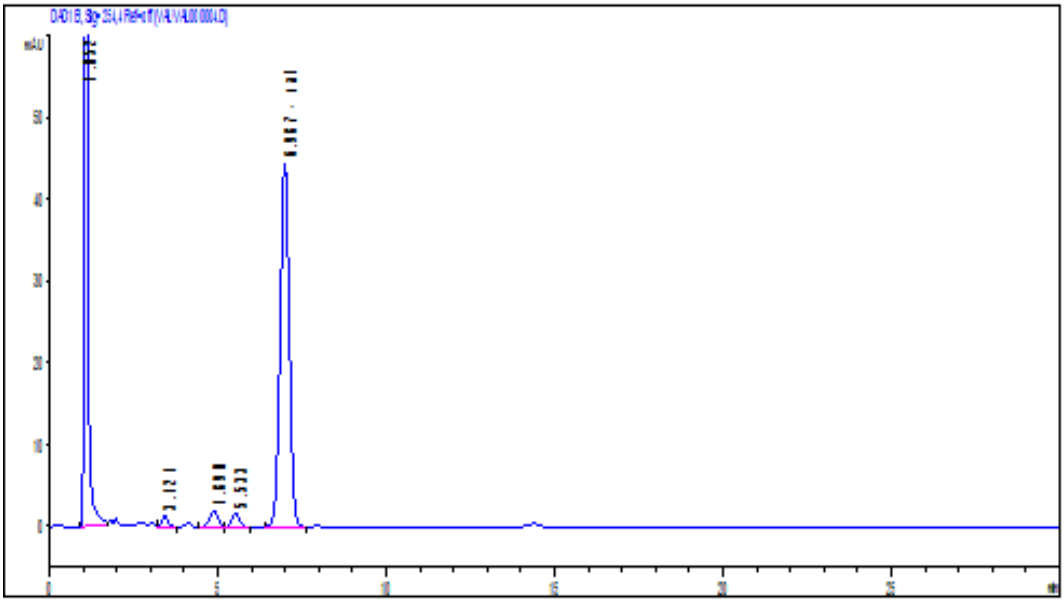

Fig 5: Valsartan after treatment with H_2O_2 7% at 60 °C for 6 hours

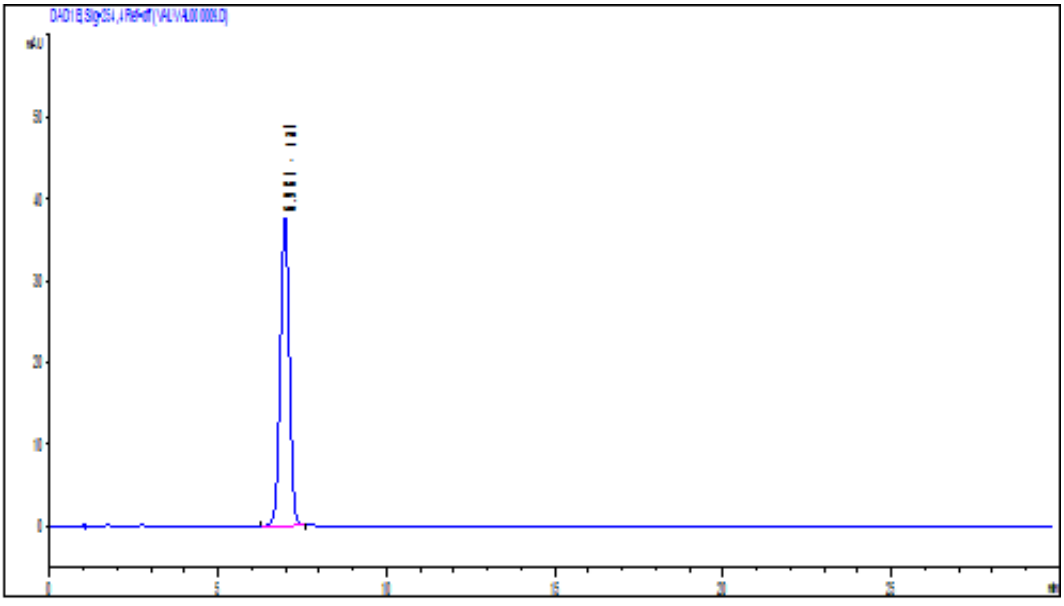

Fig 6: Valsartan after exposure to heat at 60 °C for 6 hours

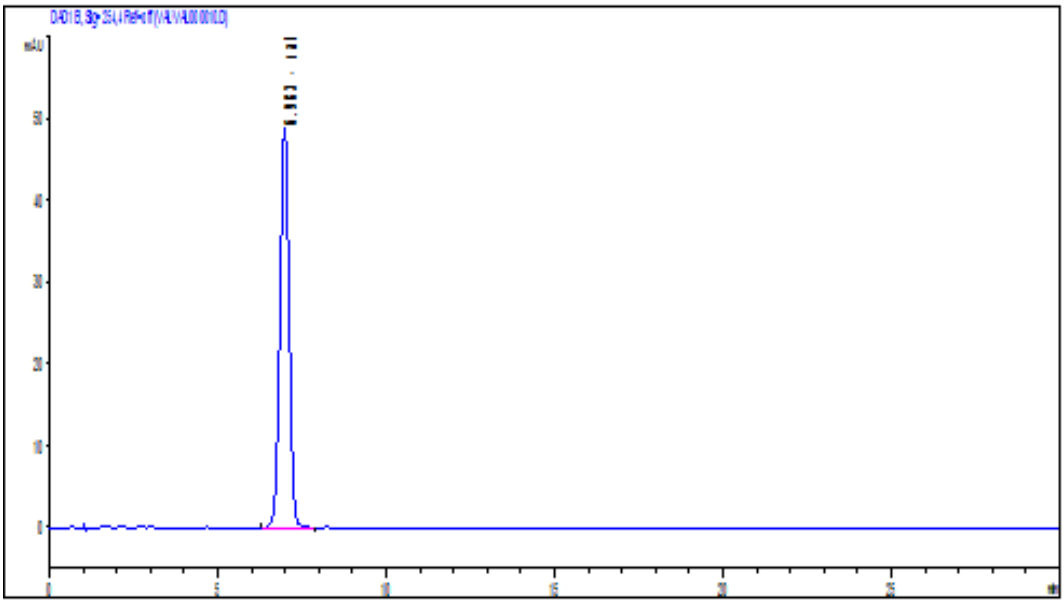

Fig 7: Valsartan after exposure to UV light for 8 hours

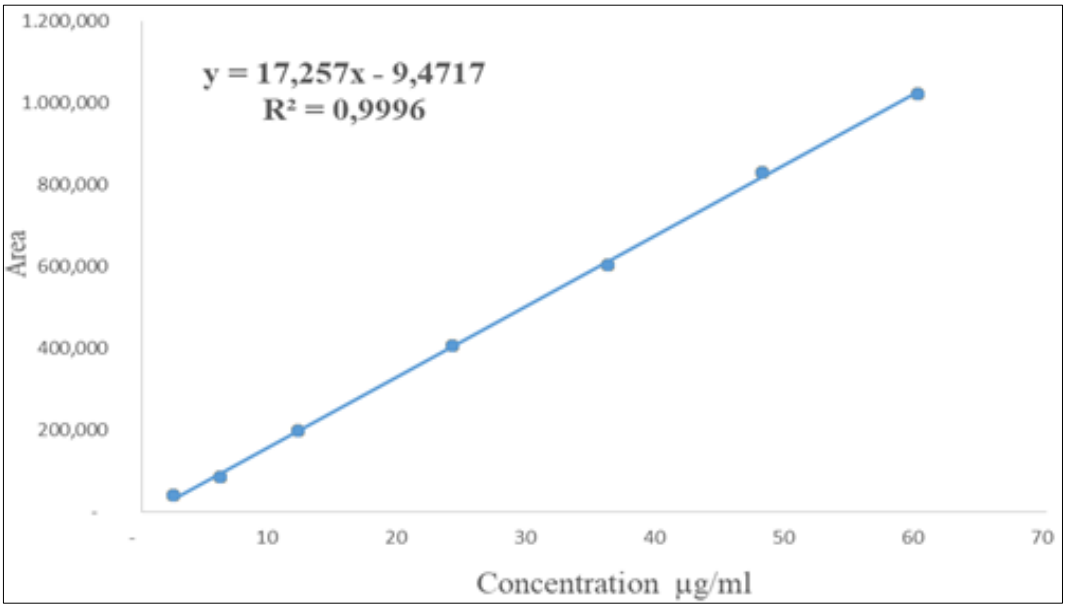

Fig 8: Linearity plot for Valsartan

Conclusions

The stability-indicating HPLC method was developed, validated according to ICH guidelines and was applied for the determination of Valsartan in tablet formulation (Table 4 and fig 2). The result obtained from validation studies revealed that, the developed method was found to be rapid, simple, accurate, precise, specific, selective and economical. Result obtained from the force degradation, indicates that in hydrogen peroxide (H_2O_2) at 60 °C and in hydrochloric acid (HCl) at 60 °C conditions drug was coTansiderably degraded. The proposed method has the ability to separate the drug from their degradation products, excipients and related substances found in tablet dosage forms and could be used as a stability-indicating method for the determination of Valsartan either in bulk powder or in tablet formulations.

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