



A Validated Stability Indicating RP-HPLC Method for Simultaneous Estimation of Valsartan and Clinidipine Combined Tablet dosage forms

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ABSTRACT

The Present Research study was developed as simple, accurate and precise stability indicating RP-HPLC method has been developed and validated for simultaneous determination of Valsartan and Clinidipine in tablet dosage forms. The chromatographic separation was carried out on an Waters column (150×4.6,i.d 5μ) with a mixture of Acetonitrile : phosphate buffer pH 3.5 adjusted with orthophosphoric acid (70:30, v/v) as mobile phase; at a flow rate of 1.0 ml/min. UV detection was performed at 254 nm. The retention times were 2.33 and 3.55 min. for Valsartan and Clinidipine respectively. Calibration plots were linear ($r^2 > 0.998$) over the concentration range 1.2-6 μg/ml for Clinidipine and 10-50 μg/ml Valsartan. The method was validated for accuracy, precision, specificity, linearity and sensitivity. The proposed method was successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that method is specific, rapid, reliable, and reproducible. The high recovery and low relative standard deviation confirm the suitability of the method for routine determination of Valsartan and Clinidipine in tablet dosage form.

Keywords: Valsartan and Clinidipine RP-HPLC; Tablet dosage forms.

INTRODUCTION

Valsartan¹⁻²: It is chemically 3-methyl-2-(pentanoyl-(4-(2-(2H-tetrazol-5-yl) phenyl) phenyl) methyl)amino)- butanoic acid (Fig. 1), angiotensin II receptor antagonist, acting on the AT1 subtype & used for treatment of high blood pressure, of congestive heart failure, and post-myocardial infarction(MI). By blocking the action of angiotensin, valsartan dilates blood pressure. The Molecular Formula- C₂₄H₂₉N₅O₃and, Molecular Weight – 435.58 .It is Soluble in various polar and Non Polar solvents like – Soluble in Acetonitrile, practically insoluble in water.

Clinidipine¹⁻²: It is chemically nominated as 1, 4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine carboxylic acid 2-methoxyethyl(2E)-3-phenyl-propenyl ester (Fig. 2). It is a dual blocker of L-type voltage-gated calcium channels in vascular smooth muscle and N-type calcium channels in sympathetic nerve terminals that supply blood vessels.

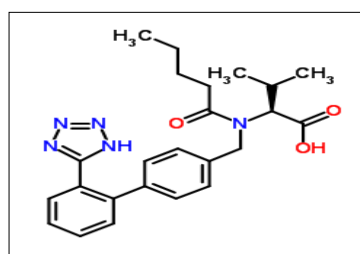


Fig.No.1.Chemical structure of Valsartan

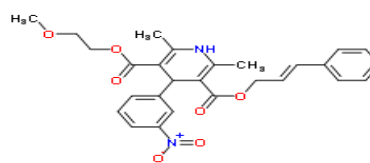


Fig. 2.Chemical structure of Cilnidipine

From the literature survey it was found that many methods are available for determination of Clindipine and Valsartan individually and there is no method was reported above combination of Valsartan and Clindipine. However, no stability indicating HPLC³⁻⁶ has been reported for simultaneous determination of Valsartan and Clindipine in combined tablet dosage forms. In the proposed study an attempt will be made to develop a stability indicating HPLC method for simultaneous estimation of Valsartan and Clindipine in pharmaceutical formulation (tablet).

EXPERIMENTAL AND RESULTS

Instrumentation: In This chromatographic tool consisted of Waters 2695 equipped with a waters C₁₈ column (250 × 4.6 mm; 5μ), 515 pumps and an2699 Photo diode array detector and auto sampler system. The output signal was monitored and integrated by Empower -2 software. Waters C₁₈ column (250 x 4.6 mm; 5μ) was used for this method. Solubility of the compound was enhanced by sonication on an ultrasonicator (PCI Analytics PCI81). All the weighings in the experiments were done with Shimadzu balance (model AX200). PVDF membrane filters were purchased from Merck Millipore.

Drugs and chemicals: Reference samples of Valsartan (VAL purity 99.9%) and Clindipine (CDP purity 99.8%) were obtained from Hetero Labs Ltd. (Hyderabad, India) as gift samples. The commercial tablet formulation, Atedio (Ajinomoto Pharmaceuticals Co., Limited) was purchased from local market. Potassium dihydrogen phosphate and orthophosphoric acid were purchased from Qualigens Chemicals Limited. HPLC grade methanol was procured from Merck Limited. HPLC grade water was prepared by using Millipore Milli-Q system.

Preparation of buffer: 7.0 g of potassium dihydrogen orthophosphate was transferred into a beaker containing 1000 mL of HPLC grade water and mixed. The pH of the solution was adjusted to 3.5 with liquid ammonia. The solution was then filtered through a 0.45μ membrane filter and sonicated.

Preparation of mobile phase: The above phosphate buffer (pH 3.5) was mixed thoroughly with acetonitrile the ratio of 30:70 v/v. This solution was used as the mobile phase.

Preparation of mixed working standard solution of valsartan and clindipine: 10 mg of Valsartan and 12 mg of clindipine were accurately weighed and transferred into two separate 10 mL volumetric

flasks. About 5 mL of acetonitrile was added in to each flask and sonicated. The volumes were made up to with further quantity of acetonitrile and mixed well. A quantity of 1.0 mL of each of the above drug solutions was transferred into a 10 mL volumetric flask and the volume was made up with the diluent to get concentrations of and 100 μg/mL and 120 μg/mL of Valsartan and clindipine respectively. This solution was used as the stock solution.

Preparation of Phosphate buffer :(p^H:3.5): Weighed 7.0 grams of Potassium Di hydrogen Ortho Phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjust Ph 3.5 with Orthophosphoric acid.

Preparation of mobile phase: Mix a mixture of above Buffer 300 mL (30%), 700 mL of Acetonitrile HPLC (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 μ filter under vacuum filtration.

Diluent Preparation: Use the Mobile phase as Diluent.

Preparation of the Valsartan & Clindipine Standard & Sample Solution:

Standard Solution Preparation: Accurately weigh and transfer 12 mg of Valsartan and 10mg of Clindipine working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3 & 0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

RESULTS AND DISCUSSION

Specificity:

Blank interference: The specificity was done by interference of placebo with analyte and the proposed method was eluted by checking the peak purity of valsartan and Clindipine during the force degradation study. The peak purity of the Valsartan and Clindipine was found to be satisfactory and under different stress conditions. There was no interference of any peak of degradation product with drug peak

Preparation of stock solution:

Accurately weigh and transfer 12 mg of Valsartan and 10mg of Clindipine working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (12ppm of Valsartan & 20 ppm of Clindipine): 0.1&0.2ml of stock

solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (24ppm of Valsartan&40ppm of Clinidipine):0.2&0.4ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (36ppm of Valsartan & 60ppm of Clinidipine): 0.3&0.6ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (48ppm of Valsartan & 80ppm of Clinidipine): 0.4&0.8ml of stock

solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (60ppm of Valsartan & 100ppm of Clinidipine) 0.5&1.0ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Procedure: Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

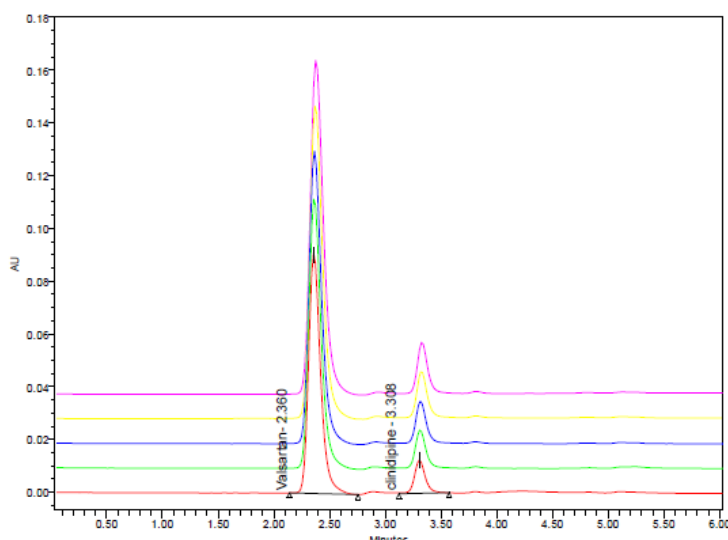


Fig.No.3.Overlain Chromatogram for mixture of Valsartan and Clinidipine .

PRECISION STUDIES:

Expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions the conditions.

Repeatability/ Intra Repeatability: (Intra--assay precision): Precision under same operating conditions over a short interval of time interval time.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration): Accurately weigh and transfer 6mg of Valsartan and 5mg of Clinidipine working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock Solution): Further pipette 0.3&0.6ml of Valsartan & Clinidipine of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% solution:(With respect to target Assay concentration): Accurately weigh and transfer 12 mg of Valsartan and 10mg of

Clinidipine working standards into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution). Further pipette 0.3&0.6ml of Valsartan & Clinidipine of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% solution:(With respect to target Assay concentration):

Accurately weigh and transfer 18mg of Valsartan and 15mg of Clinidipine working standards into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution). Further pipette 0.3 & 0.6ml of Valsartan & Clinidipine of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Intermediate precision: Precision within laboratories variation Precision variation--days, equipment, analyst equipment, analyst.

Reproducibility: Inter laboratories precision laboratories precision--technology transfer, technology transfer.

Preparation of Stock Solution: Accurately weigh and transfer 12 mg of Valsartan and 10mg of Clinidipine working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3&0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Intermediate Precision/ Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Preparation of stock solution: Accurately weigh and transfer 12 mg of Valsartan and 10mg of Clinidipine working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.3&0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

ACCURACY STUDIES

Preparation of Standard stock solution: Accurately weigh and transfer 12 mg of Valsartan and 10mg of Clinidipine working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.3&0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation Sample solutions:
For preparation of 50% solution (With respect to target Assay concentration): Accurately weigh

and transfer 6mg of Valsartan and 5mg of Clinidipine working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock Solution). Further pipette 0.3&0.6ml of Valsartan & Clinidipine of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% solution:(With respect to target Assay concentration): Accurately weigh and transfer 12 mg of Valsartan and 10mg of Clinidipine working standards into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.3&0.6ml of Valsartan& Clinidipine of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% solution:(With respect to target Assay concentration): Accurately weigh and transfer 18mg of Valsartan and 15mg of Clinidipine working standards into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.3 & 0.6ml of Valsartan & Clinidipine of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure: Inject the standard solution, Accuracy 50%, Accuracy 100% and Accuracy 150% solutions. Calculate the Amount found and Amount added for Valsartan & Clinidipine and calculate the individual recovery and mean recovery values.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

The flow rate was varied at 0.8 ml/min to 1.2ml/min: Standard solution 36 ppm of Valsartan & 60 ppm of Clinidipine was prepared and analysed using the varied flow rates along with method flow rate.

The results are summarized: On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate $\pm 10\%$.

Table No.1.The accuracy Studies

% Level		Peaks Areas		Amount Added (mg)		Amount Found (mg)		% Recovery		Mean Recovery	
VAL	CDP	VAL	CDP	VAL	CDP	VAL	CDP	VAL	CDP	VAL	CDP
50	50	1250261	90441.3	4	5	3.96	4.99	99.0	99.9	98.8	100.2
100	100	2504966	180387.3	8	10	7.93	9.96	99.1	99.6		
150	150	3732011	274315.8	12	15	11.8	15.1	98.5	101.0		

Table 2 System suitability results for Valsartan and Clinidipine

S.No	Parameter	Results	
		Valsartan	Clinidipine
1.	Retention time (Min)	2.31	3.3
2.	Resolution	-----	5.0
3.	Number of Theoretical Plates	2156.7	6302.3
4.	Tailing Factor	1.1	1.2
5.	HETP	14.37	42.01

* Results for actual flow (1.0ml/min) have been considered from Assay standard.

The Organic composition in the Mobile phase was varied from 70% to 80%: Standard solution 36 µg/ml of Valsartan & 60µg/ml of Clinidipine was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

The results are summarized: On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ± 10

Limit of Detection & Limit of Quantification: LOD & LOQ of Clinidipine was found to be 2.96, 9.97µg/ml for Valsartan was found to be 2.59, 9.95 respectively. All the system suitability parameters are within in the limits when the drugs are subjected to stress conditions like acid, base peroxide, thermal and photolysis. The results obtained were satisfactory and good agreement as per the ICH guidelines.

Specificity:

Blank interference: The prepared Blank (diluent) has been injected into HPLC as per methodology.

Acceptance criteria: Blank chromatogram should not show any peaks at the Retention times of the analyte peaks.

Observation: Blank chromatogram has not shown any peaks at the Retention times of the analyte peaks.

Intermediate precision/ ruggedness: To evaluate the intermediate precision (also known as

Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Preparation of stock solution: Accurately weigh and transfer 12 mg of Valsartan and 10mg of Clinidipine working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution): Further pipette 0.3&0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

ACCURACY STUDIES

Preparation of Standard stock solution: Accurately weigh and transfer 12 mg of Valsartan and 10mg of Clinidipine working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution): Further pipette 0.3&0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure: Inject the standard solution, Accuracy 50%, Accuracy 100% and Accuracy 150% solutions.

Calculate the Amount found and Amount added for Valsartan & Clinidipine and calculate the individual recovery and mean recovery values.

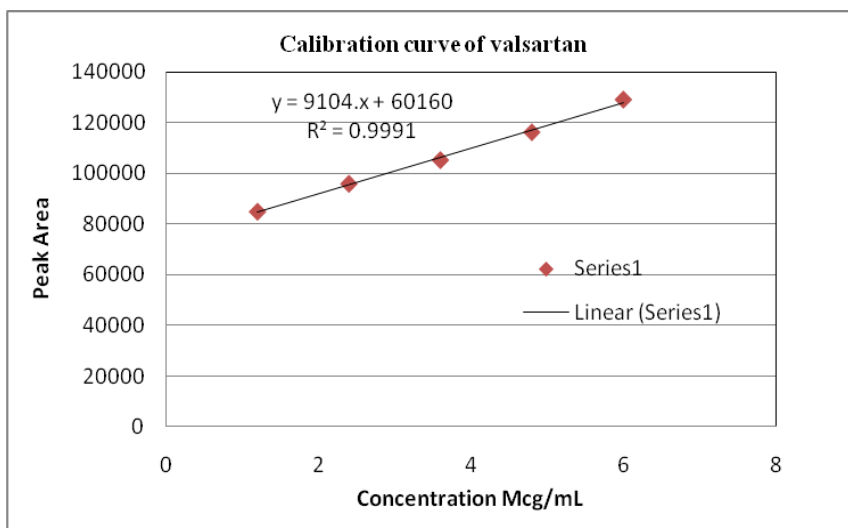


Fig.No.4. Calibration curve of Valsartan

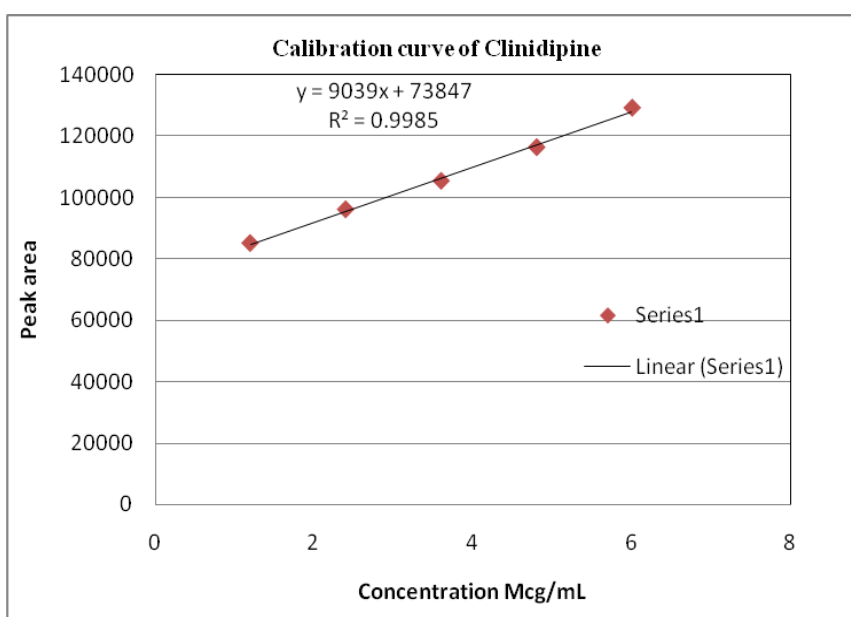


Fig.No.5 Calibration curve of Clinidipine.

Degradation study: In an order to the determine whether the analytical methods were stable Valsartan and Clinidipine combine tablets are stress on the different conditions to applied degradation studies. The guidelines are expressed in ICH Q2A, Q3B, Q2B & FDA 21 CFR section of 211 all the required for development & for the validation of stability study.

The degradation of a sample was prepared by the transfer the tablet powder was equivalent to the weight of each tablet was transfer into 100 ml flask & it was treated under the acidic, alkaline, thermal, oxidizing and photolytic conditions. When degradation was complete the solution were left to

a Equilibrate to the room temp & dil. with mobile phase to furnish the solutions of a concentration equivalent to a 15µg/mL of Clindipine and 168.25µg/mL of Valsartan. The specific degradative conditions are described below.

Acid degradation study: The Acid degradation was done by sample was treated with 3ml of 1N hydrochloric acid and kept for 10hrs at 60°C. After 10hrs the solution was neutralized with 3ml of 1N sodium hydroxide, made the volume up to the mark with mobile phase and analyzed using HPLC. The degrading drug content was found up to 4.41% in the acidic condition (Fig. 6 & 7)

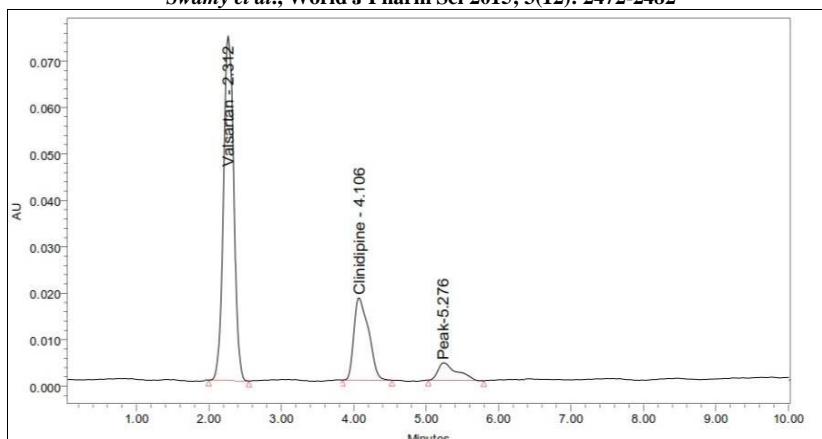


Fig 6: Chromatogram showing effect of acid degradation

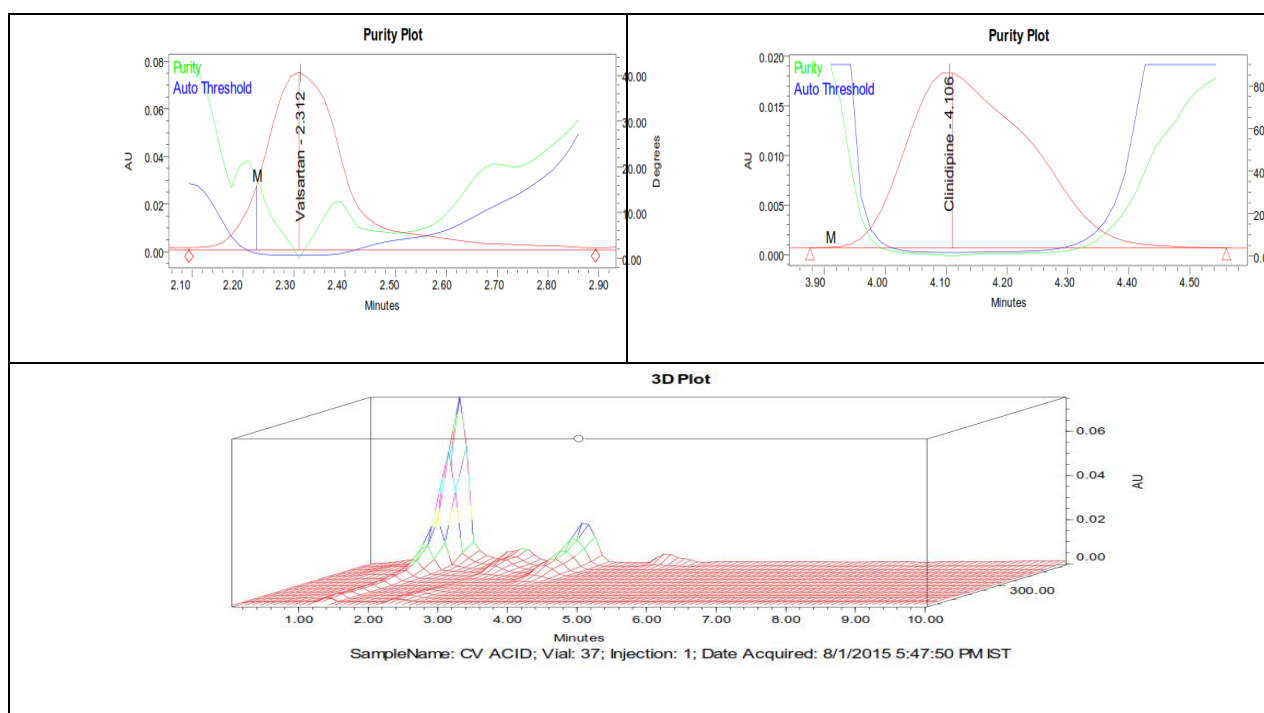


Fig. 7: Purity Plots

Alkaline degradation: The Alkaline degradation was done by sample was treated with 3ml of 1N sodium hydroxide and kept the sample for 10hr. After 10hr solution was neutralized to add 3ml of

1N hydrochloric acid, made the volume up to the mark with irrelevant media and analyzed using HPLC. In alkali degradation study, it was found to be 4.48% of the degraded drug (Fig. 8 & 9).

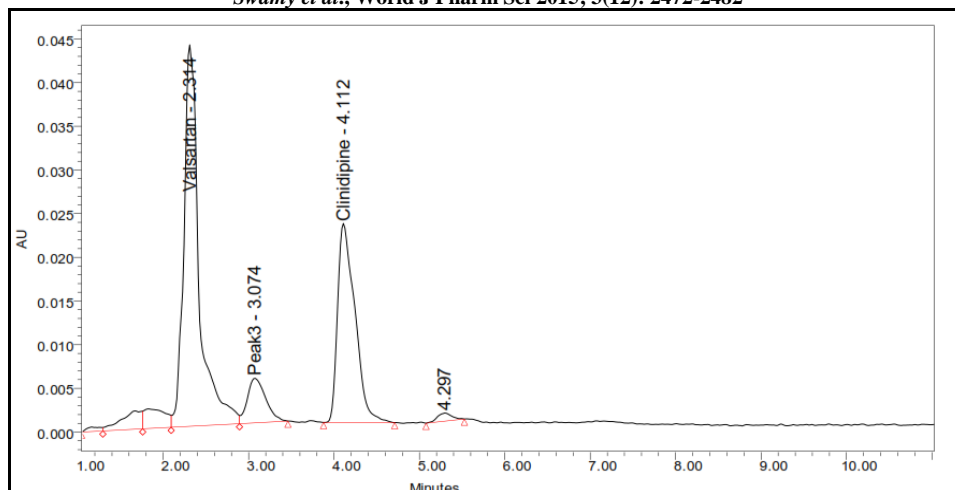


Fig. 8: Chromatogram showing effect of alkali degradation

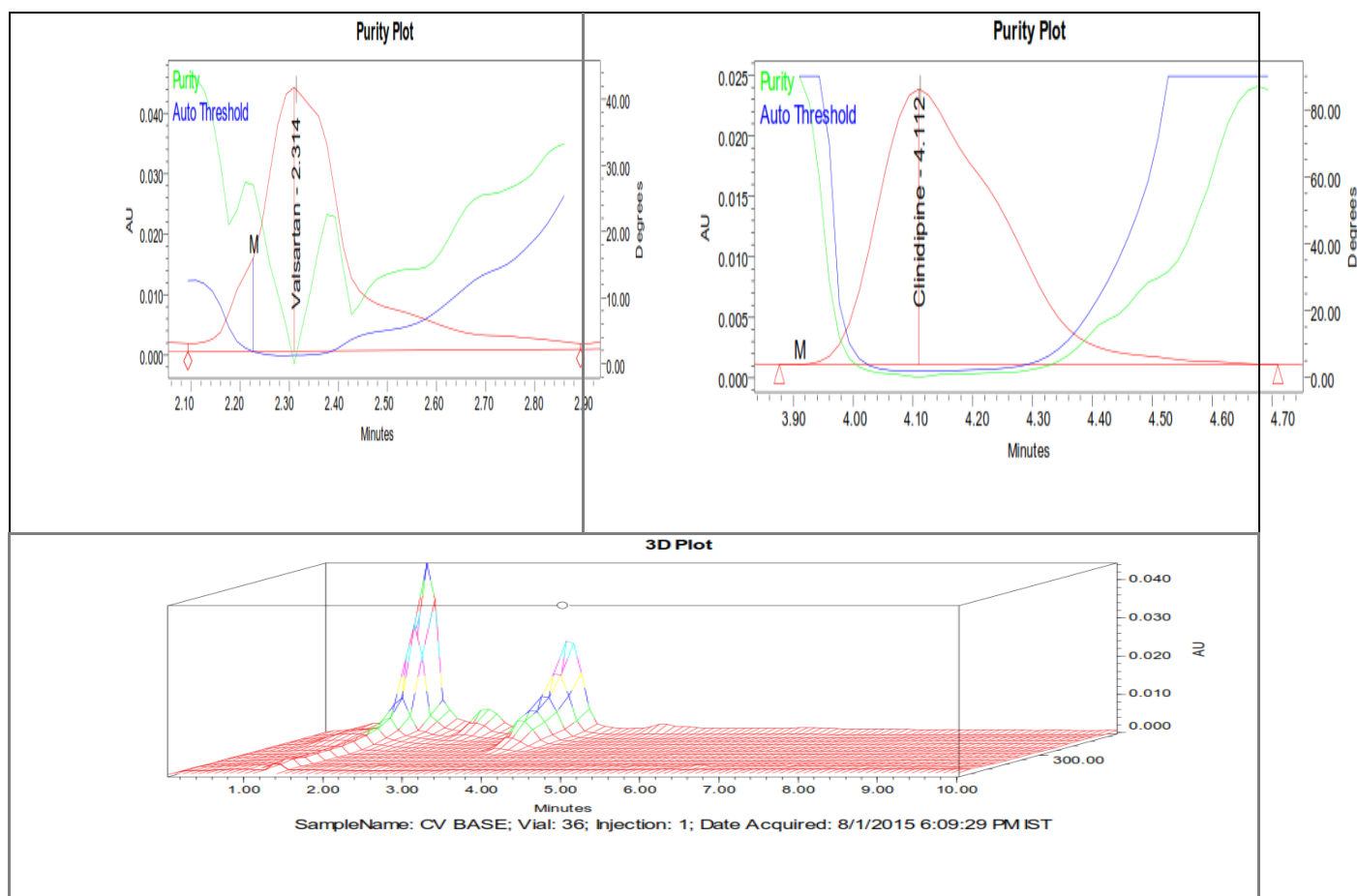


Fig. 9: Purity Plots of Valsartan and Clindipine

Oxidative degradation: The oxidative degradation was done by sample was mixed with 3mL of 30%v/v aqueous hydrogen peroxide solution and kept for 10hrs. After 10hrs made the volume upto

the mark with mobile phase and analyzed using HPLC. In oxidative degradation, it was found to be 5.41% of the degraded drug (Fig. 10 & 11).

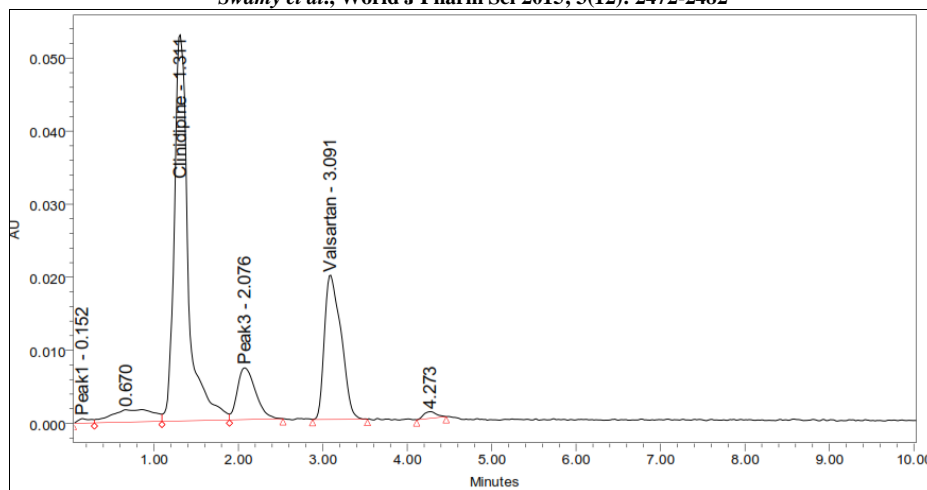


Fig. 10: Chromatogram showing effect of oxidative degradation

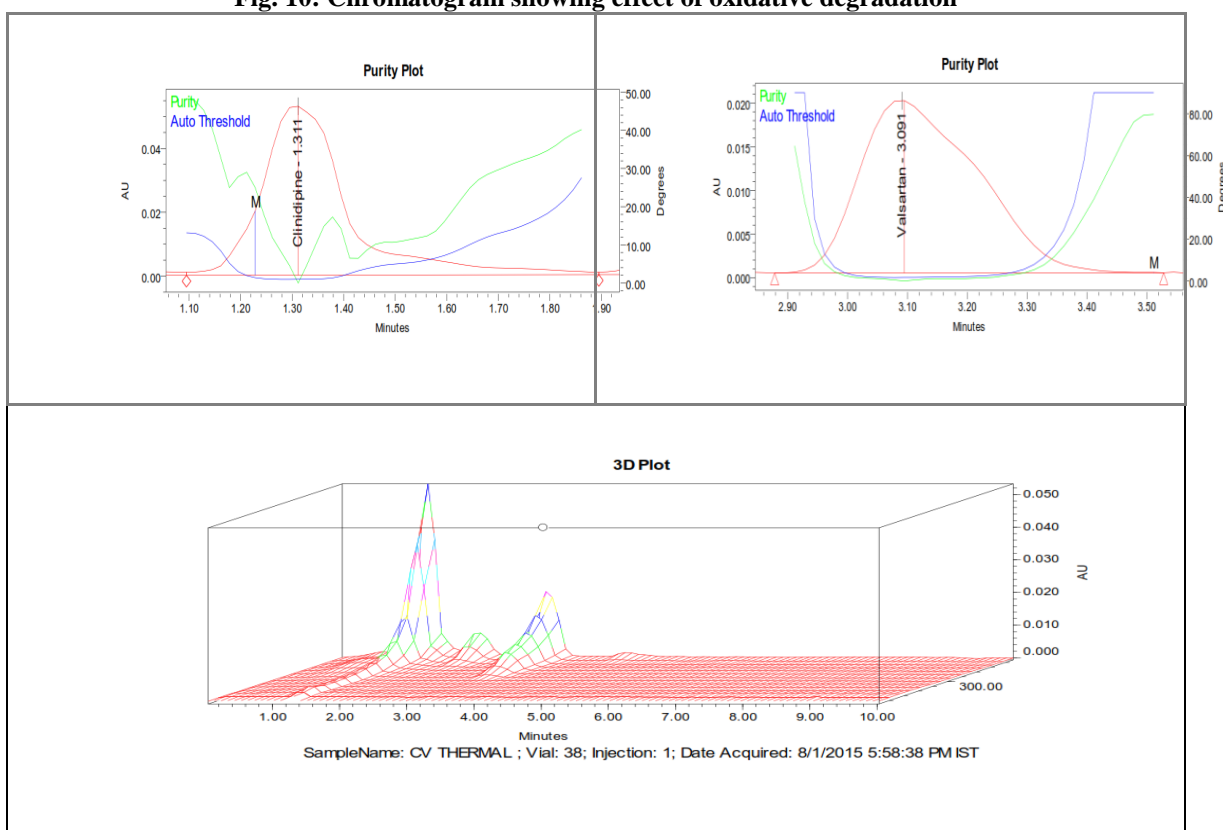


Fig. 11: Purity Plots for Valsartan and Clindipine

Photolytic degradation: The photolytic degradation was done by exposing of drug content under the UV light for 15mins to 7days. There is 6.47% of the drug degradation observed in the above specific photolytic degradation condition (Fig.12 & 13).

Thermal degradation: The Thermal degradation is to be performing by the exposing the solid drug

at the 80°C for 15mins to 60mins and at 220°C for 2-5mins. Resultant chromatogram of thermal degradation study (Fig. 14 & 15) was indicates that the drug was found to be slightly stable under thermal condition. It was only 11.20% of the drug content were degraded.

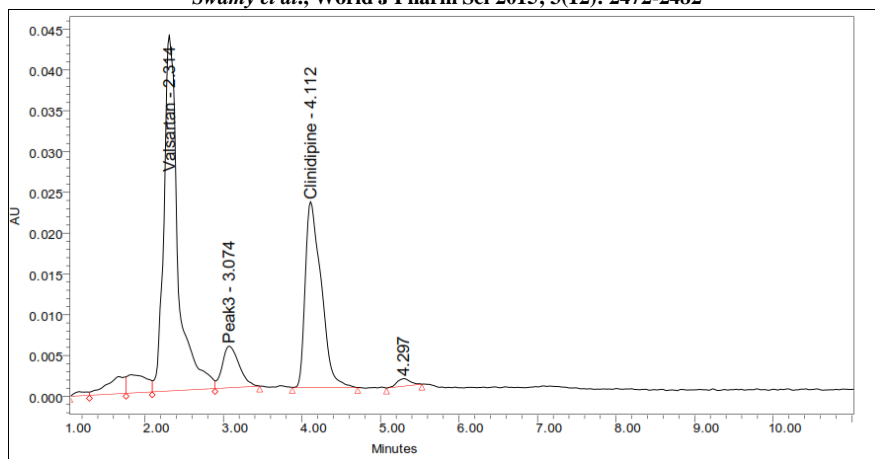


Fig. 12: Chromatogram showing effect of UV-light degradation

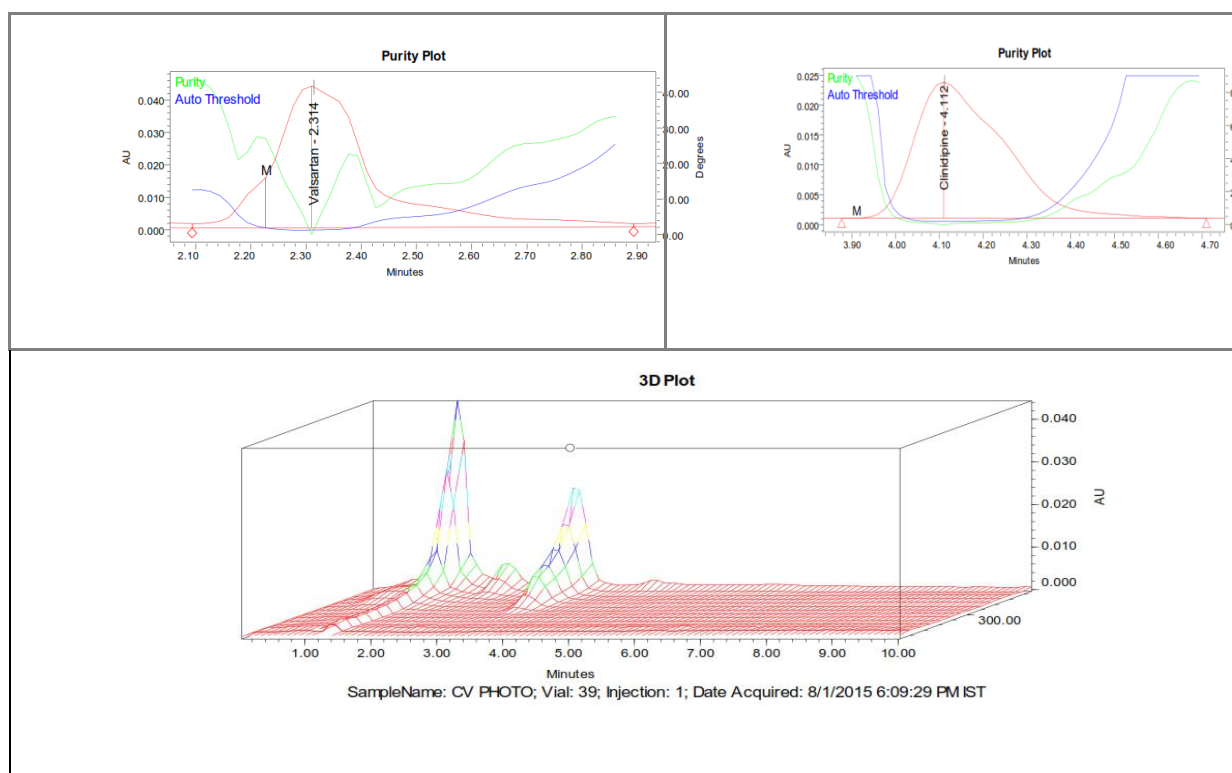


Fig. 13: Purity Plots of Valsartan and Clinidipine

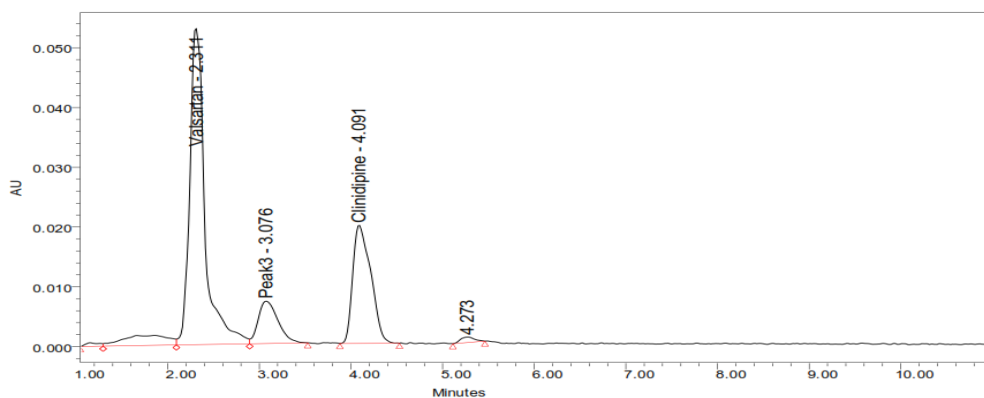


Fig. 14: Chromatogram showing effect of thermal degradation

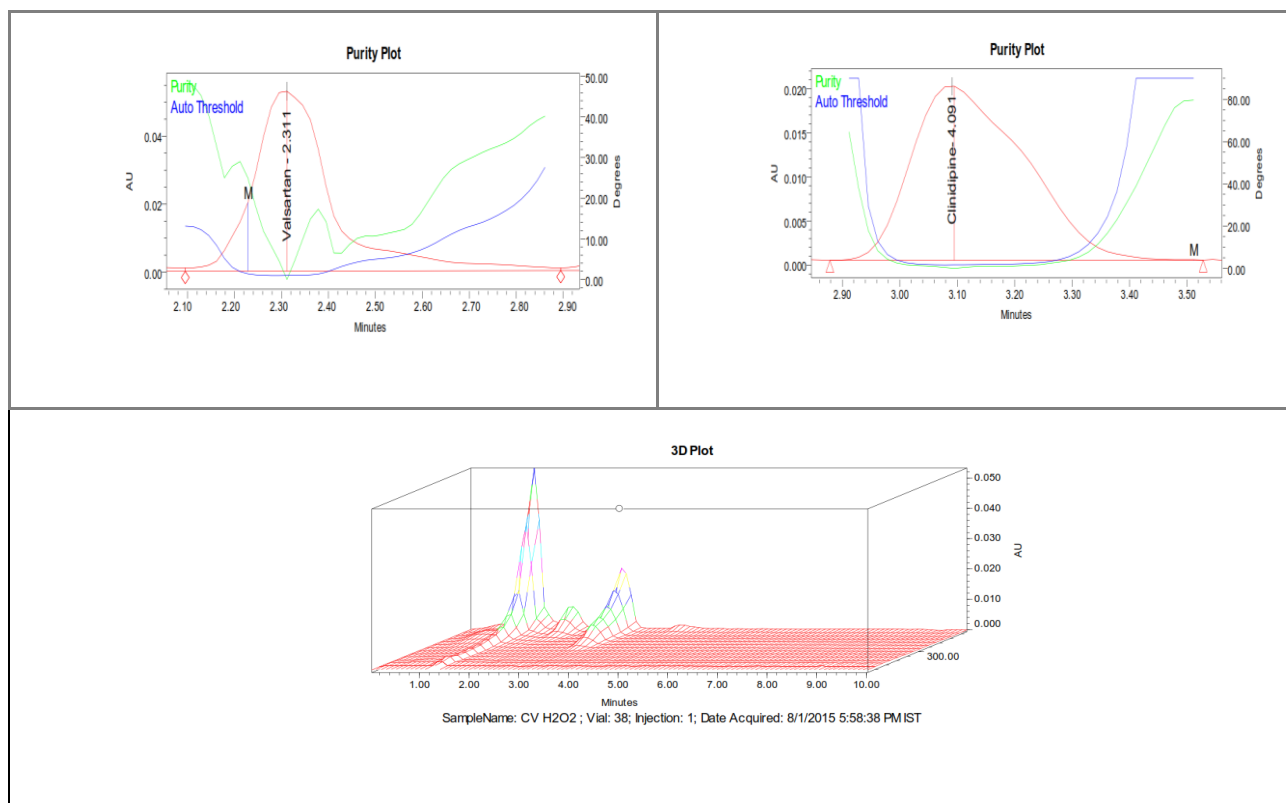


Fig No.15. Peak purity plots

Table 2: Peak purity results of Valsartan and Clindipine

Condition	Purity Angle		Purity Threshold	
	Valsartan	Clinidipine	Valsartan	Clinidipine
Acid Degradation	0.921	1.713	1.52	2.538
Alkali Degradation	0.474	1.986	2.275	3.151
Oxidative Degradation	1.741	0.013	2.467	1.731
Photolytic Degradation	0.474	1.986	2.275	3.151
Thermal Degradation	0.013	1.741	1.731	2.467

CONCLUSION

A simple, precise and accurate stability indicating RP-HPLC method is described for simultaneous determination of Valsartan and Clindipine in pharmaceutical formulations. The developed method was validated by testing its linearity, accuracy, and precision, limits of detection and quantitation and specificity. The method is simple,

fast and is without the use of ion pair or any derivatization reagent. The method is good enough to separate the peaks of active pharmaceutical ingredients (APIs) from the degradation products (produced during forced degradation studies). So, it is concluded that the method can be successfully used for any kind of stability and validation studies.

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