

Determination of Inherent Stability of Valsartan by Stress Degradation and Its Validation by HPLC

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ABSTRACT

The objective of the present study was to develop a validated stability-indicating assay method (SIAM) for valsartan subjecting it to forced decomposition under hydrolysis, oxidation, photolysis, thermal and accelerated stress conditions. Separation of drug from major and minor degradation products was successfully achieved by High Performance Liquid Chromatography (HPLC) on Kromasil C18 column utilizing, water: acetonitrile in the ratio of 60:40 with 0.5 % of ortho phosphoric acid (OPA). The detection was carried out in the range of 190-400 nm. The same major decomposition product could be seen in all the decomposed solutions. The method was validated with respect to linearity, precision (including intermediate precision), accuracy and specificity. The response was linear in the drug concentration range of 20-320 µg/ml. The correlation coefficient was found 0.9996. The % RSD values for intra- and inter-day precision studies were <1 %. The recovery of the drug from a mixture of degraded samples was ranged between 98.76 to 100.9%. PDA peak purity test confirmed the specificity of the method. The method could also be successful in analysis of drug in marketed tablets subjected to stability testing under accelerated conditions of temperature, hydrolysis, humidity, and to thermal and photolytic stress.

Key words: Stability-indicating assay method, Valsartan, HPLC.

INTRODUCTION

The International Conference on Harmonization (ICH) drug stability test guideline Q1A (R2) requires that analysis of stability samples should be done through the use of validated stability-indicating analytical methods.^[1] It also recommends carrying out of stress testing on the drug substance to establish its inherent stability characteristics and to support the suitability of the proposed analytical procedure. The stress testing encompasses the influence of temperature, humidity, light, oxidizing agents as well as susceptibility over a wide range of pH values (acidic, basic and neutral).

Valsartan is chemically N-(1-Oxopentyl)-N-[2'-(1H-tetrazol-5-yl) [1, 1'-biphenyl]-4-yl] methyl-L-valine, is a potent angiotensin receptor blocker.^[2] Literature survey revealed that HPLC^[3-5], LC-MS^[6-8], Protein precipitation^[9], Capillary electrophoresis^[10] and simultaneous UV

spectrophotometric methods^[11-12] are reported for estimation of valsartan alone or in combination with other drugs. In the present study degradation pattern of valsartan under different ICH recommended stress conditions was determined and an accurate, precise and selective reverse phase stability-indicating HPLC assay procedure for the analysis of valsartan in bulk drug samples was established.

EXPERIMENTAL

Materials

Valsartan was received as a gift sample from Ranbaxy Laboratories Ltd. (Dewas, India) and was used without further purification. Sodium hydroxide and hydrochloric acid was purchased from Ranbaxy Fine Chemicals Limited (New Delhi, India). Hydrogen peroxide was procured from Merck Ltd. (Mumbai, India). HPLC grade acetonitrile was purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical reagent grade.

Instrumentation

The pH of the mobile phase was checked on a pH/ion analyzer (Orion 420 A+, Thermo Electron Corporation (USA)). Refluxing of the drug in hydrolysis conditions was carried out in a round bottom flask-condenser assembly using

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a boiling oil bath. Stability studies were carried out in humidity chamber (Thermo Lab, Mumbai, India) and a photostability chamber (KBF 240, WTB Binder, Tuttlingen, Germany), capable of controlling tolerances in temperature (± 1 °C) and humidity (± 3 °C), below the limits specified in the ICH guideline Q1A (R2). The photo stability chamber was equipped with light sources defined under option 2 in the ICH guideline Q1B. Both UV and VIS lamps were put on simultaneously. Thermal stability study was performed in a dry air oven (Thermo Lab, Mumbai, India).

The HPLC system consisted of a 600 E pump, a 2996 photodiode array (PDA) detector, a 717 auto injector, and a degasser module; data were acquired and processed using Millennium software version 2.1 (all equipment from Waters LC Modular 2690 Separation module (USA). The chromatographic separations were carried out on Kromasil C18 Stainless steel column (250 mm x 4.6 mm, 5 μ m). Robustness testing of the method was done on another HPLC system (Shimadzu, Japan) consisted of a LC-10AT VP pump, a SPD-10A VP, PDA detector, a Phenomenex, Luna C18 (250 mm x 4.6mm, 5 μ m) column, a Phenomenex, HPLC Guard cartridge system and a Class LC10/M10A software.

Chromatographic Condition

The mobile phase consisted of acetonitrile: water (40:60 v/v, 0.5 % OPA). The mobile phase was filtered on a 0.22 μ m membrane filter and then ultrasonicated for 15 min. The flow rate was set at 1.0 ml/min. Valsartan shows good absorbance at 265 nm. A scan range of 190-400 nm was selected for stability analysis.

Degradation studies

All degradation studies in solution were carried out at a drug concentration of 1 mg/ml. Hydrolytic reactions were carried out in water, 0.1M HCl and 0.1M NaOH under refluxing for 5 days. Oxidative studies were conducted at room temperature in 3 and 30 % H₂O₂ for 24-48 h. Photo-degradation studies were carried out in water, 0.1M HCl and 0.1M NaOH in a photo stability chamber up to one month. Pure solid drug (in 1 mm thick layer in a petridish plate) was also exposed in the same chamber for one month. Suitable controls were kept under dark conditions. Pure drug was additionally exposed to dry heat at 50 °C and separately to accelerated conditions of 40 °C/75 % RH in the stability chamber for 3 months. Samples were withdrawn at suitable interval and analyzed by HPLC after suitable dilution.

Identification of major degradation products

The formation of major degradation product in reaction solutions of valsartan was checked by spiking with the standard. The suspicion that valine isomers could be formed during degradation of valsartan was based on information in literature¹³. Three unknown recurring impurities were isolated from crude valsartan by a combination of analytical and preparative liquid chromatography. One of the impurities was identified as (S)-N-valeryl-N- {[2'-(1-methyl-tetrazol-5-yl)biphenyl-4-yl]-methyl}-valine, (R)-N-valeryl-N- {[2'-(1-methyl-tetrazol-5-yl)biphenyl-4-yl]-methyl}-valine and 2-Tetrazolyl-4-methyl biphenyl by mass spectrometry and nuclear magnetic resonance spectroscopy¹³.

HPLC method development

The stressed samples were analyzed by HPLC using a Reverse Phase C18 column and a mobile phase composed of acetonitrile: water (40:60). As the separation and peak shape were not good, therefore, organic modifier concentration was added (0.5 % OPA). In this case, marked improvement was

observed. During these studies, injection volume was 20 μ l and the mobile phase flow rate was at 1 ml/min.

Validation of the method

Linearity and range

Drug solutions were prepared in the concentration range of 20-320 μ g/ml. The solutions were injected in triplicate into the HPLC column, keeping the injection volume constant (20 μ l).

Precision

Six injections, of three different concentrations (40, 160, 320 μ g/ml) were given on the same day and the values of relative standard deviation were calculated to determine intra-day precision. These studies were also repeated on different days to determine inter-day precision. Intermediate precision was established through separation studies on another chromatographic system by a different analyst.

Accuracy and Recovery

Accuracy was evaluated by fortifying a mixture of decomposed reaction solutions with three known concentrations (40, 160, 320 μ g/ml) of the drug. The recovery of added drug was also determined.

Specificity

Specificity was established by determination of purity of the drug peak using a PDA detector.

The resolution factor of the drug peak was also determined with respect to the nearest resolving peaks.

System Suitability

System suitability parameters was also determined and given in Table 1.

RESULTS AND DISCUSSION

Stress studies

HPLC studies on valsartan under different stress conditions suggested the following degradation behavior.

Acidic (0.1 M HCl) degradation

Valsartan is highly susceptible to acid hydrolysis. The drug on refluxing in 0.1M HCl resulted in formation of one major degradation product. At the end of 2 h drug degraded by almost 70 % with corresponding increase in conc. of the degradation products (Fig. 2).

Alkaline (0.1 M NaOH) degradation

Valsartan is also highly susceptible to alkaline hydrolysis. After 2 h; drug degraded by more than 90 % with corresponding increase in concentration of degradation products (Fig. 3).

Neutral (water) degradation

The drug was found stable in neutral conditions, as compared to upper two conditions falling by almost 70 % upon refluxing up to 5 days (Fig.4).

Oxidative (3% and 30% H₂O₂) degradation

The drug was found labile to oxidative degradation studies. Greater than 90 % of drug was degraded after 48 h, which was almost 70 % after 24 h with 3 % H₂O₂. With 30 % H₂O₂ drug was degraded within 24 h of study (Fig. 5).

Thermal degradation

The drug was stable at 50 °C for the study period of 3 months. There was no degradation observed during this condition (Fig. 6).

Accelerated stability study

The drug was stable at accelerated conditions of 40 °C/75 % RH in the stability chamber for 3 months. There was no degradation product observed during this stability period (Fig. 7).

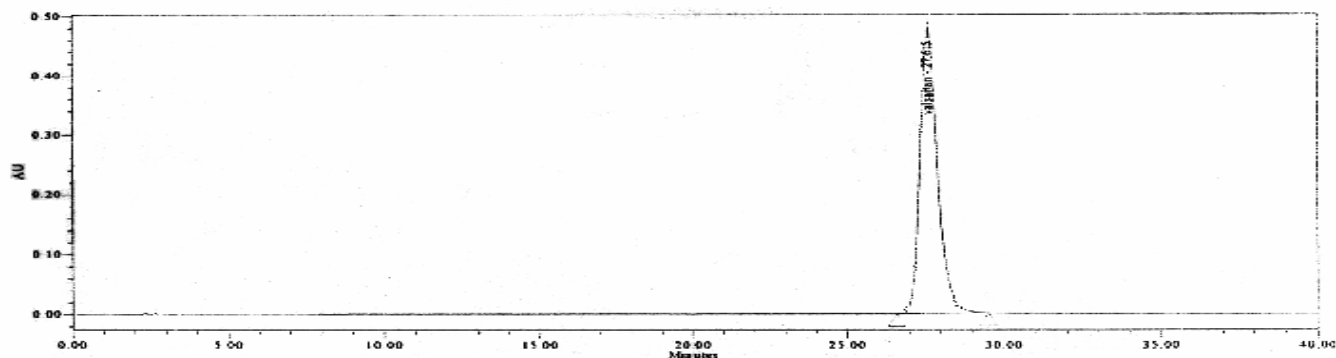


Fig. 1: Typical chromatogram of Valsartan (RT=27 ±0.5 min)

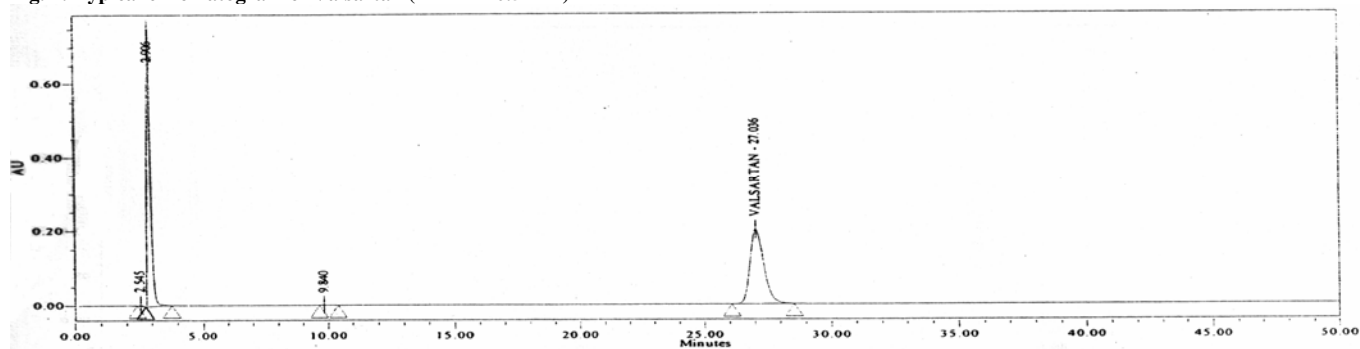


Fig. 2: Chromatogram showing degradation in 0.1 M HCl (after 2 hr)

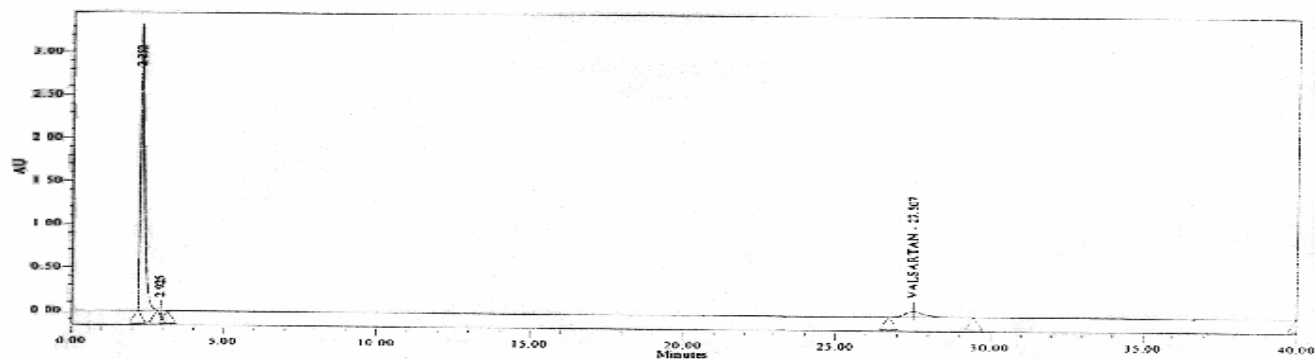


Fig. 3: Chromatogram showing degradation in 0.1 M NaOH (after 2 hr)

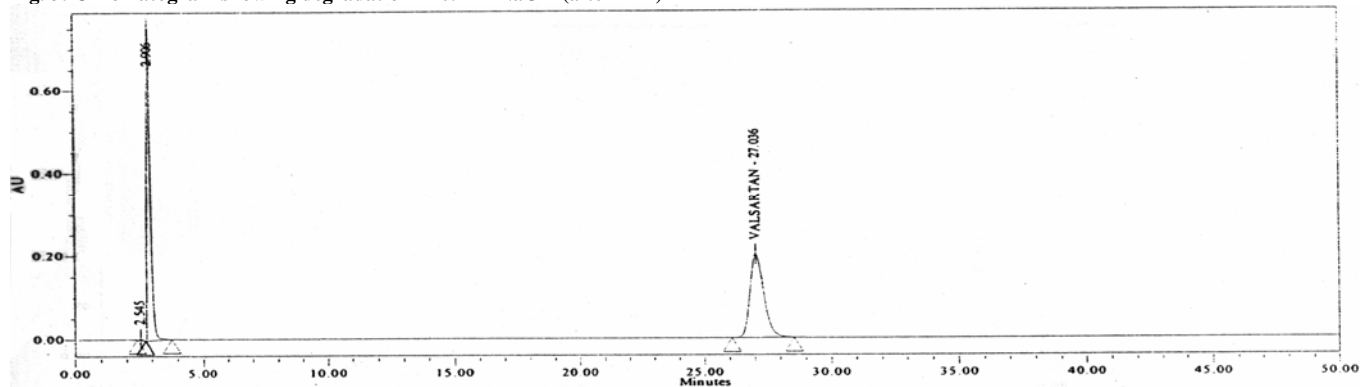


Fig. 4: Chromatogram showing degradation in Water (after 5 days)

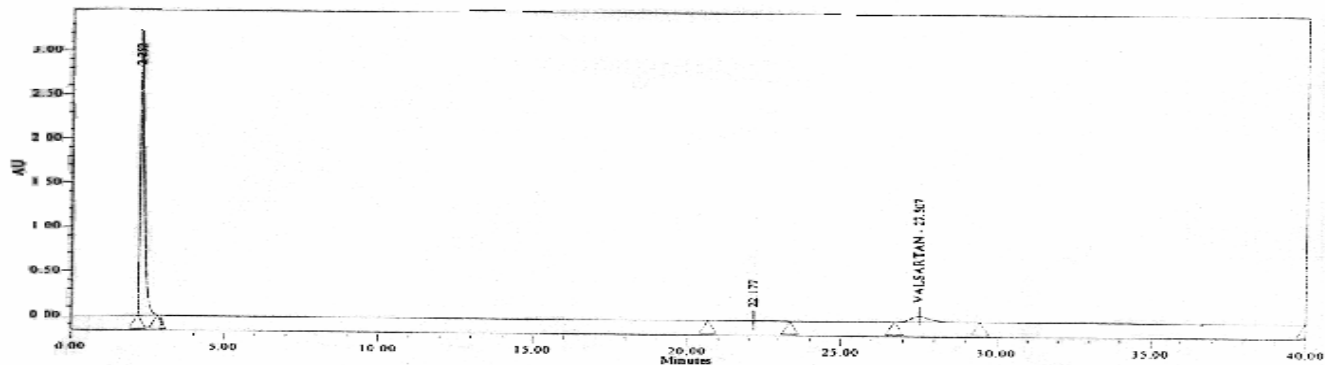


Fig. 5: Chromatogram showing degradation in 3 and 30 % H₂O₂ (after 48 hr)

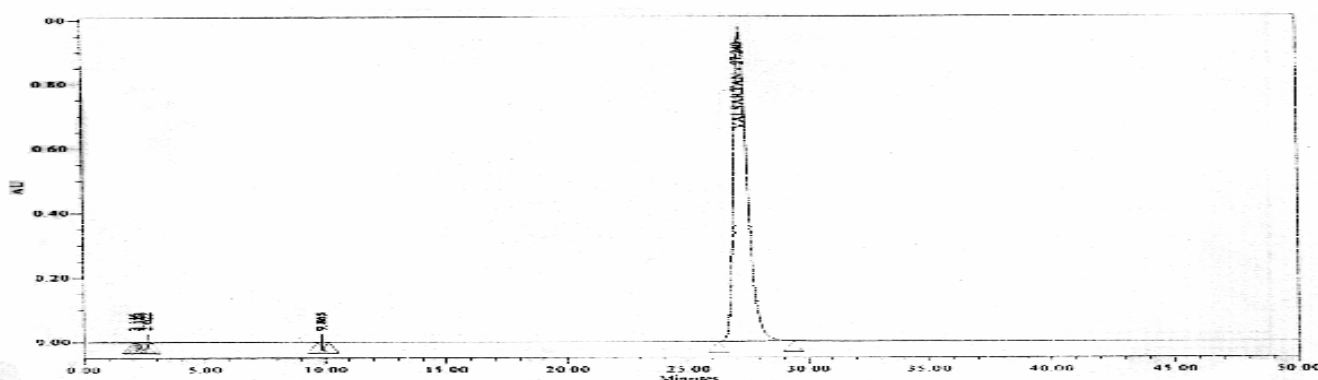


Fig. 6. Chromatogram showing degradation in Thermal condition (after 3 months)

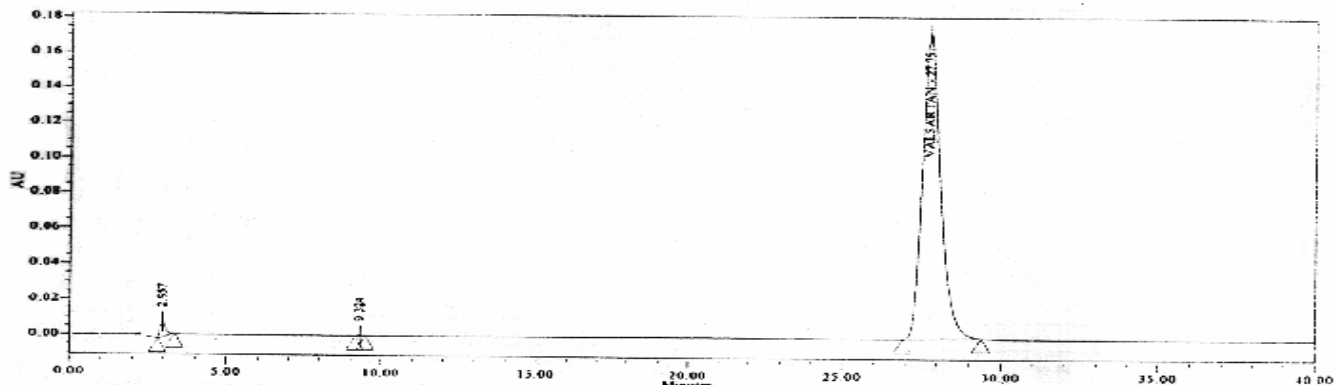


Fig. 7: Chromatogram showing degradation in Accelerated condition (after 3 months)

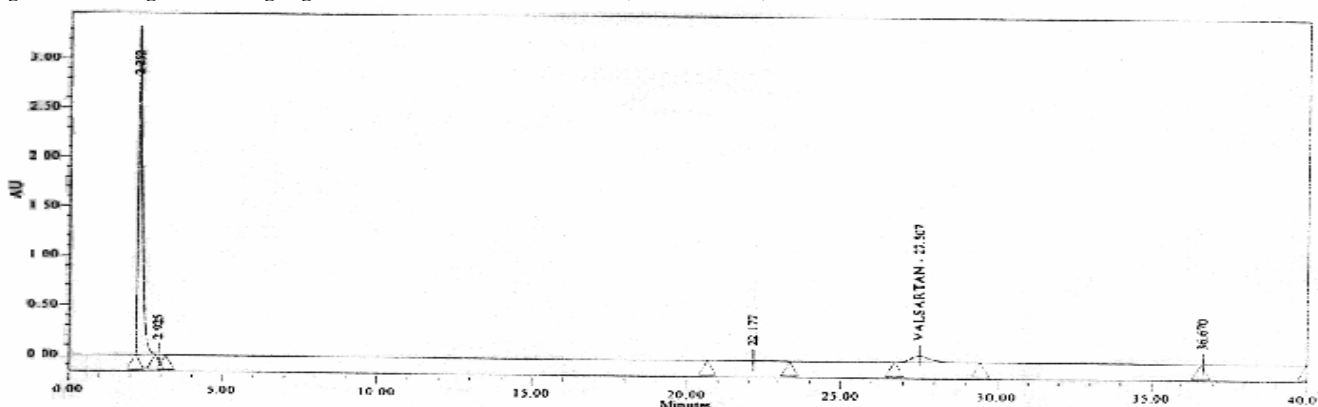


Fig. 8: Chromatogram showing degradation in Photolytic condition (after 1 month)

PARAMETERS	VALUES
Linearity range	20-320 µg/ml
Correlation coefficient	0.996
Limit of detection (LOD)	0.25 µg/ml
Limit of quantitation (LOQ)	1.0 µg/ml
Retention time (RT)	27 ± 0.5 min
Tailing factor	0.42
Theoretical plate count	7795

Table 2: Reproducibility and Precision data (Intra and Inter-day studies (n=6))

Actual concentration (µg/ml)	Measured concentration (µg/ml), % RSD	
	Intra-day	Inter-day
40	39.408, 0.835	39.254, 0.920
160	158.112, 0.652	157.102, 0.960
320	321.754, 0.988	324.263, 0.872

Table 3: Recovery studies (n = 3)

Actual concentration (µg/ml)	Calculated concentration (µg/ml); % RSD	Recovery (%)
40	39.259; 1.128	98.76
160	162.561; 1.058	101.30
320	324.073; 0.921	100.90

Photolytic degradation

Valsartan proved labile on exposure to light in acid, alkali or neutral conditions. Under all the three conditions, it decomposed to the same degradation products, also formed during hydrolysis. Rate of photolysis was highest in acid, followed by base and water (Fig. 8).

Studies on solid drug

No decomposition was seen on exposure of solid drug powder to either dry heat at 50 °C or 40 °C/75 % RH for 3 months and to the exposure in a photo stability chamber for 1 month.

Validation of the developed stability-indicating method

The drug was strictly linear in the concentration range between 20 - 320 µg/ml. The mean (% RSD) values of slope and correlation coefficient were 24256 (±0.679) and 0.9994 (±0.0265), respectively. System suitability parameters of drug were given in Table 1. Intra- and inter-day precision data are given in Table 2. The % RSD values in the two cases were <1%, which confirmed that the method was sufficiently precise. During intermediate precision testing, similar

separation behavior was observed when the method was run on another instrument by a different analyst. As shown from the data in Table 3, excellent recoveries were made at different fortified concentrations, despite the fact that the drug was added to a mixture containing degradation products. The PDA studies indicated that the method was even sufficiently specific. The purity angle value was less than the threshold angle, indicating that the drug peak was pure by nature. The resolution factors were also calculated and it was >7 for the drug peak relative to nearest resolving peak.

Applicability of the developed method to marketed formulation

The results observed from this study indicated that the method could be applied successfully even to stress marketed formulation.

A validated stability-indicating HPLC assay method was developed for valsartan, using the stress-testing route suggested by ICH. The developed method is simple, accurate, precise, specific, and could separate drug from its degradation products. It is suggested for use in analysis of samples generated during stability studies on valsartan and its formulations.

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