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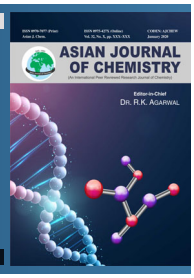

Isolation and Characterization of Novel Degradation Products of Valsartan by NMR and High Resolution Mass Spectroscopy: Development and Validation of Valsartan by UPLC

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Valsartan is used to treat high blood pressure, heart failure and diabetic kidney disease. It was subjected to forced degradation under acidic, basic and peroxide mediated oxidation. The forced degradation was performed according to ICH guidelines Q1A(R2)–Stability Testing of New Drug Substances and Products. The drug was inert under peroxide mediated hydrolysis, two degradants (DP-Val-1, DP-Val-2) were formed in base hydrolysis and one degradant (DP-Val-3) was formed in acid hydrolysis. These degradants were initially identified through LC-MS and isolated by mass mediated purification system. DP-Val-1 and DP-Val-3 are already reported but their structures were not confirmed by 2D NMR studies. DP-Val-2 is novel degradant and its structure has been elucidated by substantial analysis of high resolution mass spectrometry and 1D, 2D NMR spectroscopy. DP-Val-1 consists of a mixture of rotamers and this hypothesis was confirmed by variable temperature NMR (VT-NMR). A stability indicating RP-UPLC method was developed and validated for assay determination valsartan API drug. A valsartan UPLC method was validated on Acquity BEH C-18 2.1 × 100 mm, 1.7 µm column with shorter runtime of 6 min and the method was validated as per regulatory guidelines in terms of specificity, accuracy, linearity, precision, limit of detection and limit of quantitation.

Keywords: Valsartan, Degradation products, UPLC method.

INTRODUCTION

Valsartan is a non-peptide compound and contains a monocarboxylic acid, amide functional group and consisting of L-valine in which amino hydrogens have been replaced by a pentanoyl and a [2′-(1H-tetrazol-5-yl)biphenyl]-4-yl]methyl group and chemically described as *N*-(1-oxopentyl)-N-[[2′-(1*H*-tetrazol-5-yl) [1,1′-biphenyl]-4- yl]methyl]-L-valine. It is an angiotensin II receptor blocker, a medication used to manage hypertension, heart failure and decrease the incidence of developing adult-one set diabetes [1,2]. Forced degradation or stress degradation is the process to determine the stability of drug, formation of degradant components, preliminary kinetics and generally 5-20 % of the degradation of drug substance have been accepted for analytical method validation [3,4].

Rotational isomerism is a form of conformational isomerism, it can be interconverted by rotation about single bond. The rotational barrier is the activation energy required to covert one form of rotamer to another form, intramolecular hydrogen bonding and steric repulsion between two adjacent atoms influence the stability of rotamers [5,6]. Variable temperature (VT) NMR is preferred method to confirm the rotamers, where in NMR spectrum multiple signals were observed at room temperatures while at higher temperatures, spectrum simplifies as the equivalent peaks are averaged out [7].

There are several reports are available on valsartan stress degradation products characterisation on the basis of MS, MS/MS, 1D NMR and its validation [8-10]. However degradation product structures have not been confirmed on the basis of 2D-NMR and VT-NMR studies. In the present study, two known and one novel degradant *viz*. DP-Val-1; *N*-pentanoyl-*N*-(tetrazolo[1,5-*f*]phenanthridin-6-ylmethyl)valine, DP-Val-2; 2′-(2*H*-tetrazol-5-yl)-[1,1′-biphenyl]-4-carboxylic acid and DP-Val-3 ((2′-(2H-tetrazol-5-yl)-[1,1′-biphenyl]-4-yl)methyl)valine

were identified and their structures have been confirmed by NMR and HRMS.

Ultra performance liquid chromatography (UPLC) is a alternate technology for the HPLC analysis and has been adopted in laboratories around the world. The main advantage of UPLC system to eliminate a significant time and cost, speed, resolution and sensitivity. UPLC flow rate range 0.01-2 mL/min, back pressure up to 18000 psi and the detector high sensitive than HPLC. In the present work, UPLC analysis has been applied for the method validation, assay determination of valsartan bulk drug and reduced analysis time with good efficiency.

EXPERIMENTAL

Valsartan was a gifted sample from an API unit situated in Hyderabad, India. Chemicals and solvents used were of HPLC grade, acetonitrile (Merck), methanol (Thermo-Fisher Scientific), formic acid(Merck), trifluoroacetic acid (Merck), ammonium bicarbonate (Sigma-Aldrich), DMSO-d_6 (Cambridge isotope Limited).

High resolution mass spectrometry (HRMS): Accurate mass was measured with Q-TOF micro mass instrument equipped with micro-chanel plate detector and multimode ionization source (ES+APCI). The optimum conditions were desolvation gas flow 700 L/h, capillary voltage 3500 V, cone voltage 30 V, MCP voltage 2700 V, positive ionisation mode. Leucine Enkephalin (556.2771 Da) was used to calculate Lteff and the elemental compositions were calculated with Masslynx(4.1) software.

H-Class UPLC instrument: H-Class UPLC equipped with quaternary solvent manager and 2996 PDA detector was used for method validation. Method conditions were column: Acquity UPLC BEH C–18 2.1 × 100 mm 1.7 µm, mobile phase: (A) 0.05 % triflouroacetic acid; B: 0.05 % triflouroacetic acid in acetonitrile with gradient time (%) of B 0/40, 3/98, 5/98, 6/40; flow rate: 0.3 mL/min; column temp.: 30 ºC.

LC-MS instrument: Waters SQD-2 mass instrument coupled with UPLC, Column: Acquity BEH C-18 2.1 × 50 mm 1.7 µm, mobile phase A: 0.075 % formic acid; mobile phase b: 0.075 % formic acid in acetonitrile, gradient time (%) of A 0/95, 2.5/2, 3.5/2, 4/95; column temp. 35 ºC, flow rate: 0.5 mL/min; cone volatage: 30 V, capillary voltage 2800 V; Nebulization gas 750 L/h.

Mass mediated purification system: Preparative HPLC coupled with 2545 binary gradient module, 2767 sample manager, 515 make up pump and SQ Detector-2. Column: Inertsil ODS-3, 5 µm 20 × 250 mm, mobile phase A: 0.01 M ammonium bicarbonate, mobile phase B: acetonitrile; gradient time (%) of B 0/30, 11/90, 11.5/98, 13/98, 15/30; flow rate 19 mL/min, detection at 248 nm.

NMR analysis: Valsartan degradation impurities were recorded on Bruker 500 MHz resolution instrument with top spin software, DMSO-d_6 solvent was used for all the experiments and TMS used as internal standard.

Stress methods: The stress conditions *viz*. acid, base hydrolysis and oxidation were carried out as per ICH guideline, A 0.5 N HCl was used for acid hydrolysis and refluxed for 5 h and the formation of degradant percentage was very low and the reflection is extended to 24 h. A 1N NaOH was used for base catalyzed hydrolysis and refluxed for 24 h and 30 % H_2O_2 was used for peroxide mediated oxidation. The major degradants were identified in acid and base hydrolysis.

RESULTS AND DISCUSSION

The degradants were identified after 5 h of reflux condensation, however, percentage formation was very less, thus refluxed was continued to 24 h. The percentage of conversion was monitored through LC-MS, where 1 mL of drug reaction mass was diluted with mobile phase and injected 2 µL in the LC-MS system. Two major degradants identified in base hydrolysis and one degradant identified from acid hydrolysis (Fig. 1). The identified degradants were taken for isolation.

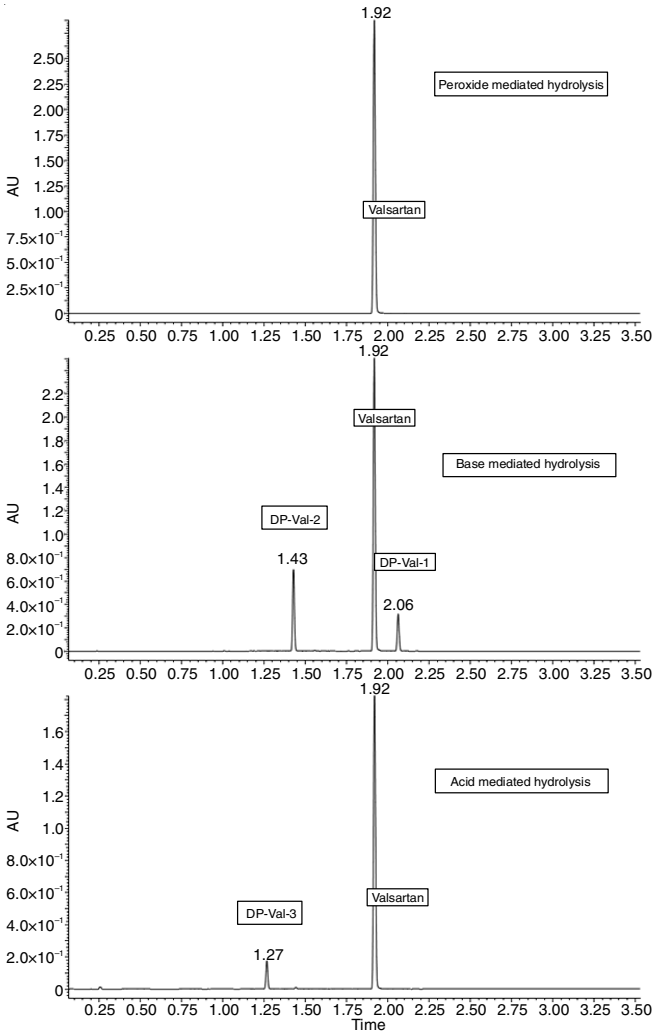

Fig. 1. Degradation chromatogram of valsartan

Isolation of acid and base degradation products: The degradation products were isolated through mass mediated preparative HPLC. The degradants were separated through preparative column, identified with mass and the resulting fractions were collected separately and lyophilized. Identified degradant products were assigned as DP-Val-1, DP-Val-2 and DP-Val-3 (Fig. 2).

Structure elucidation of DP-Val-1: The mass spectrum of DP-Val-1 shows protonated molecular ion peak at 434.2195 $[M+H]^+$ and protonated molecular formula $C_{24}H_{28}N_5O_3$ was confirmed by HRMS spectrum (Fig. 3).

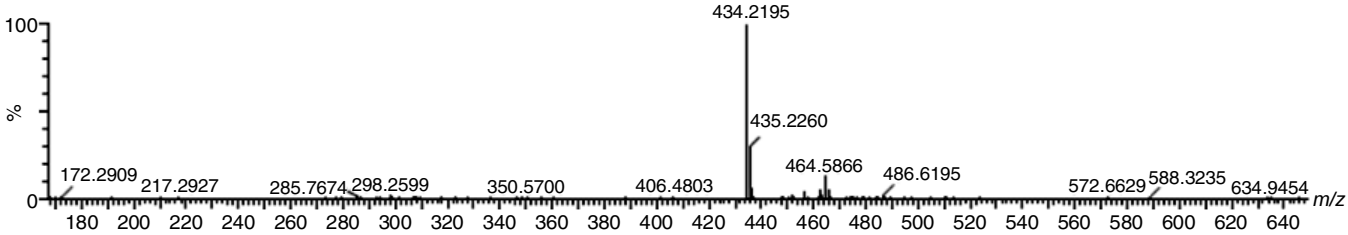

Fig. 3. HRMS spectrum of DP-Val-1

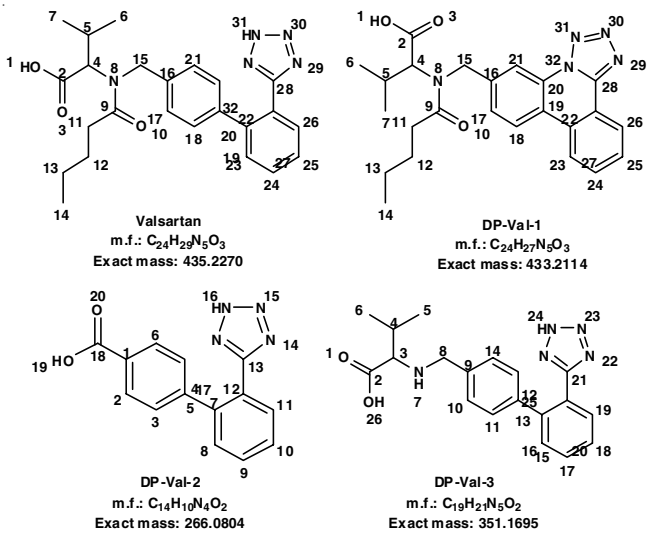

Fig. 2. Proposed structures for valsartan degradation products

^{1}H NMR spectrum of DP-Val-1 at room temperature revealed a duplication of signals, which suggested the presence of two rotamers in solution, similarly variable temperature (VT) ^{1}H NMR studies also supports this hypothesis. One set of ^{1}H NMR signals were observed at 100 ºC indicates that DP-Val-1 possesses rotamers and this was due to *N*-pentanoyl linkage of DP-Val-1 and also exihibited 1,2,4-trisubstitution benzene pattern protons instead of 1,4-disubstituted benzene protons of valsartan drug. DP-1 had total seven aromatic protons and nineteen aliphatic protons while ^{13}C NMR data revealed that DP-1 had 15 aromatic carbons and 9 aliphatic carbons. ^{13}C NMR data also showed two set of signals at room temperature due to rotamers. In COSY experiment, H-21(8.37, 8.53 ppm) correlated with H-17(7.64, 7.74 ppm). H-17(7.64, 7.74 ppm) correlated with H-21(8.37, 8.53 ppm) and H-18(8.68, 8.81 ppm) (Fig. 4).

These correlations in COSY and H-21 (8.37, 8.53 ppm), H-17 (7.64, 7.74 ppm, δ, 8 Hz), 18(8.68, 8.81 ppm, δ, 8 Hz) protons chemical shift values, multiplicity and coupling constant values supported that DP-Val-1 had 1,2,4-trisubstituted benzene pattern. It is because of cyclic six membered ring formation

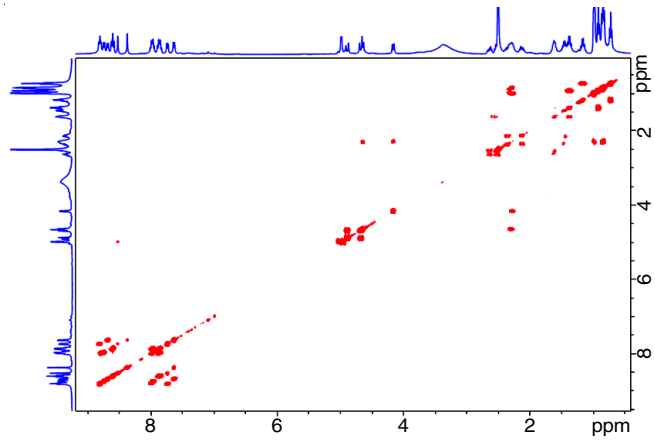

Fig. 4. COSY spectrum of DP-Val-1

happened due to bond formation between C-20 (benzene ring) and N-32 (tetrazole ring). HSQC analysis provided the information that it had 7 aromatic methines, 2 aliphatic methines, 4 aliphatic methylene and 3 methyl groups.

Structure elucidation of DP-Val-2: A protonated molecular ion peak of DP-Val-2 was observed at 267.0892 $[M+H]^+$ and protonated molecular formula $C_{14}H_{11}N_4O_2$ with below 5 ppm error was confirmed by HRMS spectrum (Fig. 5).

^{1}H NMR spectrum revealed that DP-Val-2 had 8 aromatic protons, out of 4 protons having 1,4-disubstituted benzene pattern and remaining 4 protons having 1,2-disubstituted benzene pattern. In COSY experiment, H-2,6 (7.77 ppm) correlated with H-3,5 (7.18 ppm), H-11 (7.63 ppm) correlated with H-10 (7.41 ppm), H-10 (7.41 ppm) correlated with H-9 (7.41 ppm) and H-11 (7.63 ppm), H-9 (7.41 ppm) correlated with H-10 (7.41 ppm) and H-8 (7.35ppm) as shown in Fig. 6.

In HMBC experiment, H-2,6 (7.77 ppm) correlated with 18th position carbonyl carbon at 167.6 ppm, C-4 (146.4ppm) and C-2,6 (128.5 ppm) (Fig. 7). This main key proton *versus* carbon correlation in HMBC and proton *versus* proton correlations in COSY supports the structure of DP-Val-2 as shown in Fig. 2.

Structure elucidation of DP-Val-3: The mass spectrum of DP-Val-3 shows protonated molecular ion peak at 352.1774

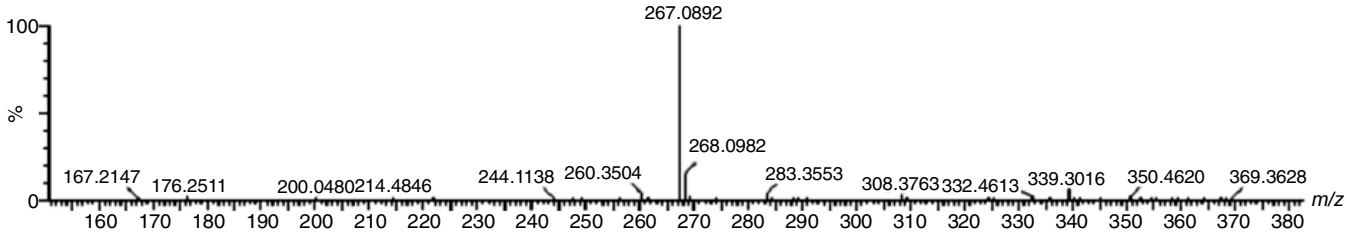

Fig. 5. HRMS spectrum of DP-Val-2

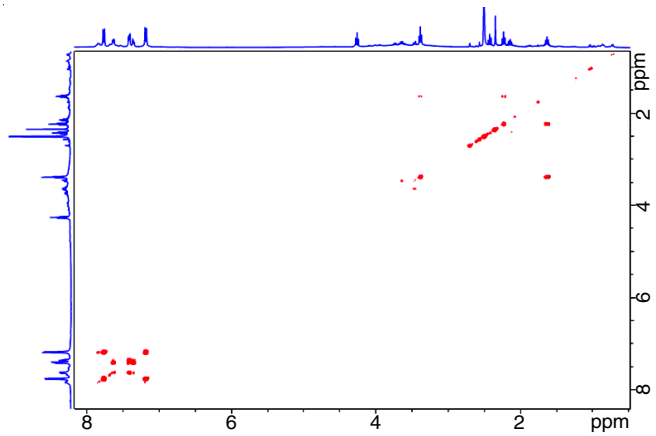

Fig. 6. COSY spectrum of DP-Val-2

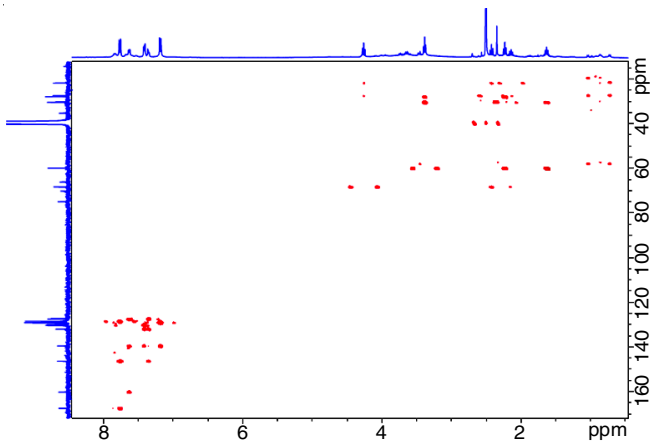

Fig. 7. HMBC spectrum of DP-Val-2

$[M+H]^+$ and protonated molecular formula $C_{19}H_{22}N_5O_2$ was confirmed by HRMS spectrum with below 1 ppm error (Fig. 8).

^1H NMR analysis shows that DP-Val-3 had 8 aromatic protons and 10 aliphatic protons. ^{13}C NMR spectra also revealed 14 aromatic carbons and 5 aliphatic carbons. In HSQC experiment, 8 aromatic methine protons, 2 aliphatic methine protons, 1 methylene group and 2 methyl groups in DP-Val-3 were observed, while in HMBC experiment, H-8 (3.80, 3.95 ppm) correlated with C-3 (66.3 ppm), C-9 (133.6 ppm) and C-10,14 (128.5ppm) (Fig. 9). These main key protons *versus* carbon correlation in HMBC supports the structure of DP-Val-3 as shown in Fig. 2.

Method development and validation: UPLC method with 6 min run time method was developed and validates as per regulatory guidelines in terms of precession (intra & inter-day), limit of detection and quantitation. Linearity was performed with 25, 50, 75, 100, 125 and 150 % of the sample, accuracy and the recovery experiments were conducted to determine the

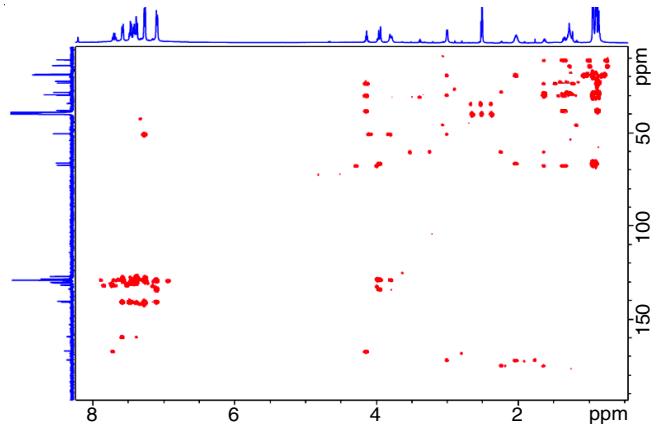

Fig. 9. HMBC spectrum of DP-Val-3

accuracy of the method. Accuracy was proved by spiking 10 % of standard solution to 50, 100 and 150 % of the sample.

Valsartan standard solution (0.3 mg/mL) was injected in the UPLC system for system suitability test, the retention time of the valsartan was 2.73 min, while USP tailing and plate count values were 1.12 and 50437, respectively. Intra-day and inter-day precession methods was checked with six repeated concentration preparations and the results are shown in Table-1.

TABLE-1
VALIDATION PARAMETERS OF VALSARTAN

Intraday method precision (n = 6, % of RSD)	0.3
Interday method precision (n = 6, % of RSD)	0.2
LOD-LOQ	
Limit of detection (mg/mL)	0.0009
Limit of quantification (mg/mL)	0.003
Precision at LOQ (% R.S.D.)	0.7
Linearity	
Calibration range (mg/mL)	0.075-0.450
Calibration points	6
Correlation coefficient	0.999

The detection limit and quantitation were 0.0009 mg/mL (S/N 4.84) and 0.003 mg/mL (S/N 16.64), respectively and the valsartan linearity was demonstrated with the concentration ranging 0.075-0.450 mg/mL, while the correlation coefficient was greater than 0.999. The accuracy and recovery of assay of valsartan was 99.24 % and the results are shown in Table-2.

TABLE-2
ASSAY RECOVERY OF VALSARTAN

Level (%)	Amount added (µg/mL)	Amount recovered (µg/mL)	Recovery (%)
50	160.08	158.14	98.78
100	200.10	200.41	100.15
150	300.05	296.45	98.80

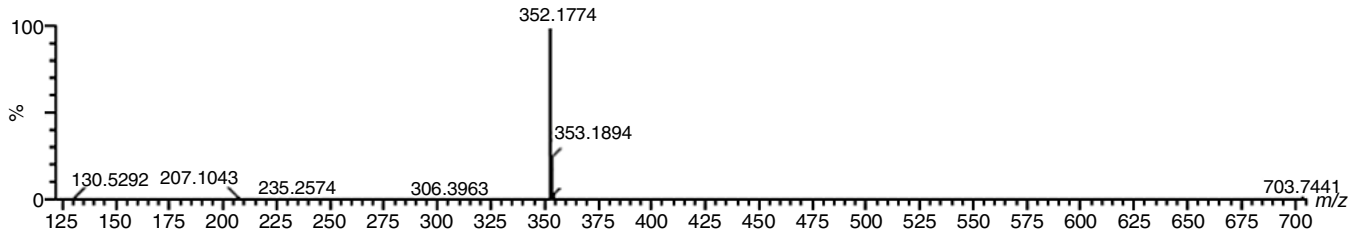

Fig. 8. HRMS spectrum of DP-Val-3

Method robustness was checked by changing the organic solvent composition (± 0.2 mL/min), pH (± 0.2), column temp. (± 5 ºC) and different systems. No illustrious changes were observed and the stability of valsartan drug solution was checked at precise temperature (2-8 ºC) for the period of 40 days, mobile phase stability was checked after 2, 4 and 7 days with valsartan drug solution and no significant changes were observed.

Conclusion

Three major degradation products (DP-Val-1, DP-Val-3) and DP-Val-2) were identified in valsartan during base catalyzed hydrolysis and acid mediated hydrolysis. These impurities were separated with mass mediated purification technique and characterized by using NMR and HRMS. The newly developed shorter UPLC method for assay determination of valsartan drug substance was faster, superior and the method was completely validated showing satisfactory data for all tested validation parameters. Thus proposed method is a stability indicating method and suitable for faster analysis of valsartan bulk drug.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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