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Determination and validation of valsartan and its degradation products by isocratic HPLC

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Abstract: Valsartan was subjected to different ICH prescribed stress studies. The stability-indicating assays were established by using isocratic RP-HPLC separation C_{18} column (250 mm length×4.6 mm internal diameter and 5 µm particle size) for both major degradants of valsartan by acid hydrolysis and by oxidation. The mobile phase comprising of methanol:water (70:30v/v, pH 7.2) was used in acid hydrolysis stability-indicating assay and the mobile phase comprising of methanol:water (60:40v/v, pH 7.2) was used in oxidation stability-indicating assay of valsartan. The flow rate was adjusted to 1.2 ml/min and detection was performed at 250 nm using a UV detector in both assays. The pure impurities were characterized by spectral studies. The degradants appeared at relative retention time (RRT) of 0.40 min and 0.27 min for acid hydrolysis and oxidation of valsartan, respectively. The validation studies established a linear response of the drug and satisfactory results for precision and recovery studies without interference with detection of valsartan. The LC-MS studies show *m/z* values of the peaks as 307.40 and 335.41. The pure impurities were isolated by appropriate route at laboratory scale. Prior to spectroscopic characterization of impurities, they were separated and purified using pH partitioning and/or extraction recrystalization and/or chromatographic techniques. A simple, precise, and accurate isocratic reversed-phase stability-indicating high performance liquid chromatographic assay method was developed and validated for determination of valsartan. Also identification of degradants was carried out and probable structures were confirmed.

Keywords: Degradation; degradant; HPLC; stability-indicating assay; LC-MS; Valsartan

1. Introduction

The revised parent drug stability test guideline Q1A (R2) issued by the International Conference on Harmonization (ICH) requires that stress testing on the drug substance should be performed to establish stability characteristics and to support the suitability of the proposed analytical method. It is suggested that stress testing should include the effect of temperature, light, and oxidizing agents. It is also recommended that sample stability should be determined through the use of a validated stability testing method. Valsartan,(S)-N-valeryl-N-[2□Œ-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl] valine(VAL), is a potent, highly selective, orally active, specific angiotensin II receptor

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antagonist used as a hypotensive drug[1-3].There is one chiral centre in the valine moiety of the molecule but the pure (S)-enantiomer is essentially used. The assigned (S)-configuration is defined from the synthetic origin ((L)-valine). Gas chromatographic, mass spectroscopic and numbers of high-performance liquid chromatographic (HPLC) methods are available for separation and quantitation of VAL from biological fluids[4-6]. This paper deals with the forced degradation of VAL under conditions such as acid hydrolysis, base hydrolysis, oxidation, thermal and UV stress. The aim of the current study was to develop a validated stability-indicating high-performance liquid chromatographic (HPLC) assay method for determination of VAL and isolation of degradation products. Method validation was done according to ICH guidelines[7].

2. Experimental

Experimental

2.1. Materials

VAL was supplied as gift sample by Lupin ltd. (Mumbai, Maharashtra, India) and used without further purification. Indapamide was supplied as gift sample by Glenmark ltd. (Goa, India) Sodium hydroxide and hydrochloric acid (both AR grade) were purchased from LOBA chemie ltd. Hydrogen peroxide was procured from SD Fine chem ltd. Methanol (HPLC grade) was purchased from Merck ltd. Double distilled water was obtained from a water distillation unit. Analytical grade triethylamine and ortho phosphoric acid were purchased from LOBA chemie ltd.

2.2. Instrumentation

The HPLC system used was a computer based Jasco series instrument comprising of a pump PU-2080 and a UV detector UV-2070. Manual injections were carried out using a Rheodine injector with a fixed 20 µl external loop. The chromatographic separations were performed on a HIQ sil C18 ODS column (250 mm lengt×4.6 mm internal diameter, 5 µm particle size), operating at ambient temperature, using a mobile phases consisting of methanol:water [70:30v/v, (pH 7.2, adjusted by adding 0.2 M ortho phosphoric acid into 0.2 M triethyl amine)] and methanol:water [60:40v/v, (pH 7.2, adjusted by adding 0.2 M ortho phosphoric acid into 0.2 M triethyl amine)] at a flow rate of 1.2 ml/min, and detection was performed at 250 nm using a UV detector. HPLC instrument was controlled by software Borwin. The mobile phase was filtered through 0.45µm nylon membrane filter. A shimadzu AY 120 analytical balance was used for weighing. A PCi ultrasonicator was used for sonication. The calibrated glasswares were used throughout the experiment. The mobile phase was used for dilutions of degradation samples throughout the analysis. Precision water baths were used for degradation studies under acidic and alkaline conditions. Dry bath was used for thermal stress studies. UV degradation was carried out in a UV chamber equipped with a light bank consisting of two UV and fluorescent lamps. LC-MS studies were carried out in negative electro spray ionization (ESI) mode on Varian Inc, USA., 500 MS IT with 410 Prostar Binary LC (Direct Infusion Mass with ESI and APCI Negative and Positive mode ionization, mass ranging from 50 to 2000 m/e) instrument.

2.3. Conduct of stress studies

The stress studies were carried out under the conditions of dry heat, hydrolysis, oxidation, and UV degradation, as defined by ICH[8].For thermal stress testing, the drug powder was sealed in glass ampoules and heated in dry bath at 500C for 8 hrs. Acid decomposition was carried out in 0.1N HCl at drug strength of 1mg/ml. As sufficient decomposition was not observed, the drug was additionally refluxed at a concentration of 1mg/ml in 1N HCl at 700C for 1 hr. The studies in alkaline conditions were conducted similarly at a drug concentration of 1mg/ml in 0.1N NaOH. These solutions were refluxed at 700C for 1 hr. After cooling at room temperature, the solutions were neutralised separately.

The oxidative stress studies were conducted at drug strength of 1mg/ml in 3% H2O2. The solution was stored at room temperature for 24 h. As sufficient decomposition was not observed, the drug was additionally exposed at a concentration of 1mg/ml in 30% H2O2 at room temperature for up to 24 h. The UV degradation studies were carried out in solid state by spreading a thin layer of drug in a petri-dish and exposing it directly to the combination of UV and florescent light. A parallel set was kept in dark under similar conditions. Samples were withdrawn after 24 hrs.

2.4. Separation studies

Before injecting to HPLC, the volume of each reaction solution was made to 100 ml with methanol. The reaction solutions were individually subjected to HPLC studies. The studies were conducted using a mobile phases composed of methanol:water [70:30v/v, (pH 7.2, adjusted by adding 0.2 M ortho phosphoric acid into 0.2 M triethyl amine)] for acid hydrolysis stability-indicating assay and methanol:water [60:40v/v, (pH 7.2, adjusted by adding 0.2 M ortho phosphoric acid into 0.2 M triethyl amine)] for oxidation stability-indicating assay of VAL. The separations were achieved by changing the mobile phase composition as well as the flow rate. The overall objective here was to develop a selective stability indicating assay method (SIAM)[9].

2.5. Validation of the developed stability-indicating method

Validation of the optimised HPLC method was done with respect to various parameters, as required under ICH guideline Q2(R1)[10]. To establish linearity and range, a stock solution of the drug was prepared at strength of 1mg/ml, which was further diluted to prepare solutions in the drug concentration range of 5-45 µg/ml and 20-100 µg/ml separately. The solutions were injected in triplicate into the HPLC column, keeping the injection volume constant (20 µl). Precision of the method was studied by making six injections of three different concentrations, viz., 10, 25 and 40 µg/ml from concentration range 5-45 µg/ml in mobile phase methanol:water [70:30v/v, (pH 7.2, adjusted by adding 0.2 M ortho phosphoric acid into 0.2 M triethyl amine)] for acid hydrolysis stability-indicating assay. Also the precision of another method was studied by making six injections of three different concentrations, viz., 30, 60 and 90 µg/ml from concentration range 20-100 µg/ml in mobile phase methanol:water [60:40v/v, (pH 7.2, adjusted by adding 0.2 M ortho phosphoric acid into 0.2 M triethyl amine)] for oxidation stability-indicating assay of VAL. For both the methods the precision studied on the same day and the values of relative standard deviation (% RSD) were calculated to determine intra-day precision. These studies were also repeated on different days to determine inter-day precision. Accuracy was evaluated by fortifying a mixture of degraded solutions with known concentration of, viz., 10 and 30 µg/ml of drug for degradation solution of acid hydrolysis and degradation solution of oxidation, respectively. The recovery of the added drug was determined. The specificity of the method was established through study of resolution factors of the drug peak from the nearest resolving peak, and also among all other peaks.

2.6. Application of the developed method to stability samples

The developed method was found to apply even to real stability samples, which was verified through successful analyses of VAL, which had been stored at accelerated conditions of temperature and humidity for 3 months before analysis. The stability indicating assay method was developed to stability samples.

2.7. Characterization of degradation product(s)

LC-MS studies were carried out to determine *m/z* values of the major degradants formed under various stress test conditions. The obtained values were compared with the molecular weights of probable degradants by acid hydrolysis and oxidation of VAL. The structures were confirmed by characterization through MS-MS data.

2.8. Isolation of impurities

The VALAD (degradation product of valsartan by acid hydrolysis) was synthesised in sufficient a quantity by acid hydrolysis of VAL. Hydrolysis was carried out using 5 ml of 1N HCl per milligram of VAL. The solution was refluxed at 700C for 1 hr. After cooling at room temperature, it was neutralised. The isolation of VALAD as impurity was done by pH partitioning. The oxidative VALO (degradation product of valsartan by oxidation) impurity was also synthesised using 30% H2O2. The reaction mixture was stored at room temperature for 24 h. The VALO was isolated by column chromatography. The synthesised impurities were characterised by using IR, NMR and mass spectra. The IR and NMR shows values as 3355 (NH stretching);3010 (CH stretching); 1610 (C=N stretching), 1590 (N=N stretching) and 1.01 (s, 6H, CH-(CH3)2); 2.0 (s, 2H, NH2)2.07 (q, 1H, CH-(CH3)2); 2.51 (d, 2H, CH-CH2-NH); 3.81 (s, 2H, NH-CH2-Ar); 7.00-7.65 (m, 7H, Aromatic) for VALAD respectively. The IR and NMR shows values as 3445 (NH stretching); 2990 (CH stretching); C=O (), 1655 (C-N stretching), 1610 (C=N stretching), 1590 (N=N stretching) and 0.98 (d, 3H, CH3); 1.61 (t, 2H, CH3CH2); 2.18 (d, 2H, CH2-CH2); 2.90 (s, 3H, N-CH3); 4.46 (s, 2H, N-CH2-Ar); 7.02-7.54 (m, 7H, Aromatic) for VALO, respectively. Mass spectra in the negative electron spray ionization (ESI) mode for the degradants were shown *m/z* values of the peaks 307.40 and 335.41 which matched 2-methyl-N-{[2'-(1H-tetrazol-5-yl)biphenyl-3-yl]methyl}propan-1-amine and N-methyl-N-{[2'-(1H-tetrazol-5- yl) biphenyl-3-yl] methyl} phenamide as degradant of VAL by acid hydrolysis and degradant of VAL by oxidation, respectively.

3. Results and discussion

The retention times (RT) and relative retention times (RRT) of the drug and the degradation products are listed in Table IA and Table IB. The data obtained from linearity studies are given in Table IIA and Table IIB. The representative of calibration curves are shown in Figure 1 and Figure 2. The average % recovery of the drug was observed 101.10 and 103.05 from degradation solutions of acid hydrolysis and oxidation of VAL, respectively, confirming that the method was sufficiently precise. The precision data are given in Table IIIA and Table IIIB. Good separation was achieved even when the procedure was repeated by a different person, thus confirming the reproducibility of the method. As shown from the data in Table IV and Table V, good recoveries were made at the added concentration of 10 and 30 µg/ml of drug for degradation solution of acid hydrolysis of VAL and degradation solution of oxidation of VAL, respectively. The average % recovery of the drug was observed 101.10 and 103.05 from degradation solutions of acid hydrolysis and oxidation of VAL, respectively. Figure 3 and Figure 4 shows that the method was selective to the drug as well as the degradation products. The system suitability parameters are given in Table VI and Table VII.

Table 1a. Retention time and relative retention times of various peaks

Peak	Retention time (RT)	Relative retention time (RRT)
VAL	9.57	1.00
VALAD	3.86	0.40

VAL: Valsartan; VALAD: Valsartan acid degradant

Table 1b. Retention time and relative retention times of various peaks

Peak	Retention time (RT)	Relative retention time (RRT)
VAL	8.06	1.00
VALO	2.24	0.27

VALO: Degradant of valsartan by oxidation; VAL:valsartan

Table 2a. Linearity data on three different days by using mobile phase methanol:water [70:30v/v, (pH 7.2)]

Regression parameter	Day 1	Day 2	Day 3	Mean±SD(% RSD)
Slope	0.1418	0.1418	0.1420	0.1418±0.0001(0.07052)
Intercept	-0.0487	-0.0481	-0.0602	-0.0523±0.0068(13.001)
R2	0.9994	0.9994	0.9991	0.9993±0.0001(0.0100)

Table 2b. Linearity data on three different days by using mobile phase methanol:water [60:40v/v, (pH 7.2)]

Regression parameter	Day 1	Day 2	Day 3	Mean±SD(% RSD)
Slope	0.0617	0.0617	0.0612	0.0615±0.0002(0.3252)
Intercept	0.2155	0.0265	0.0750	0.0412±0.0298(72.3300)
R2	0.9992	0.9992	0.9990	0.9991±0.0001(0.0100)

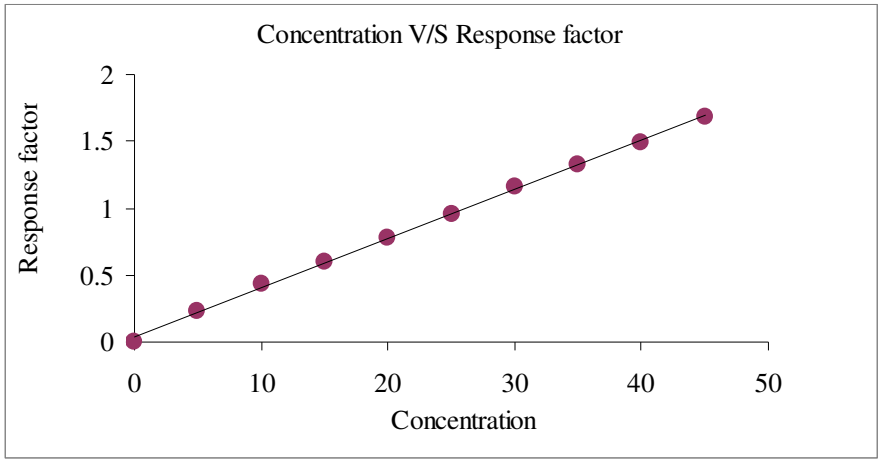

Figure 1. The representative calibration curve showing linearity data [mobile phase methanol:water (70:30v/v), pH 7.2 -Day 1]

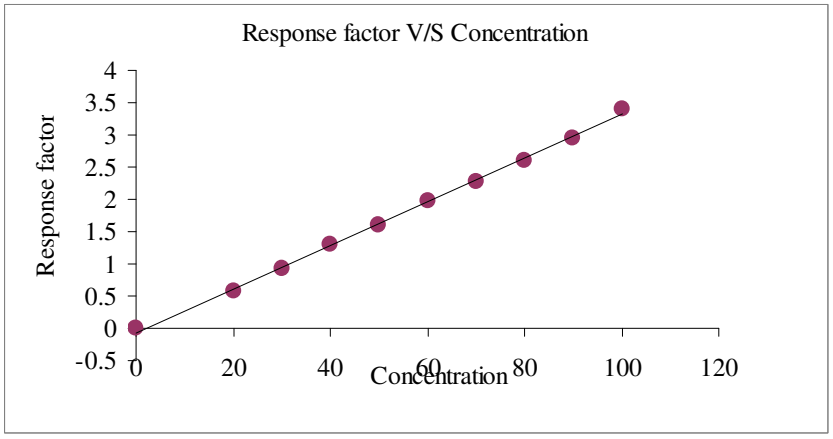

Figure 2. The representative calibration curve showing linearity data [mobile phase methanol:water (60:40v/v), pH 7.2 -Day 1]

Table 3a. Reproducibility and precision data obtained during intra-day (n=6) inter-day (n=3) studies by using mobile phase methanol:water [70:30v/v, (pH 7.2)]

Actual Concentration (µg/ml)	Intra-day measured concentration (µg/ml)±SD (% RSD)	Inter-day measured concentration (µg/ml)±SD (% RSD)
10	09.94±0.1437(1.4456)	09.89±0.3485(3.5237)
25	24.58±0.5075(2.0646)	24.94±0.1654(0.6631)
40	39.65±0.4732(1.1934)	39.98±0.5583(1.3964)

Table 3b. Reproducibility and precision data obtained during intra-day (n=6) inter-day (n=3) studies by using mobile phase methanol:water [60:40v/v, (pH 7.2)]

Actual Concentration (µg/ml)	Intra-day measured concentration (µg/ml)±SD (% RSD)	Inter-day measured concentration (µg/ml)±SD (% RSD)
30	30.21±0.8562(2.8341)	30.05±0.5255(1.7487)
60	59.74±0.3069(0.5137)	59.83±0.6242(1.0432)
90	89.61±0.5448(0.6079)	89.95±0.5391(0.5993)

Table 4. Recovery studies using nine different dilutions from degradation solution (acid hydrolysis of valsartan)

Volume of acid degradation solution diluted to 10 ml	Actual added pure drug concentration (µg/ml)	Measured of pure drug concentration (µg/ml)	Recovery (%)
0.4	10	10.02	100.2
0.6	10	09.98	99.80
0.8	10	10.02	100.20
1.0	10	09.87	98.70
1.2	10	09.86	98.60
1.4	10	10.02	100.20
1.6	10	10.01	100.10
1.8	10	09.85	98.50
2.0	10	09.96	99.60
	Average recovery		99.54

Table 5. Recovery studies using nine different dilutions from degradation solution (oxidation of valsartan)

Volume of oxidation degradation solution diluted to 10 ml	Actual added pure drug concentration (µg/ml)	Measured of pure drug concentration (µg/ml)	Recovery (%)
0.2	30	29.67	98.70
0.3	30	30.12	100.40
0.4	30	30.24	100.80
0.5	30	29.78	99.26
0.6	30	30.25	100.83
0.7	30	30.45	101.50
0.8	30	30.35	101.16
0.9	30	30.23	100.76
1.0	30	29.95	99.83
	Average recovery		100.36

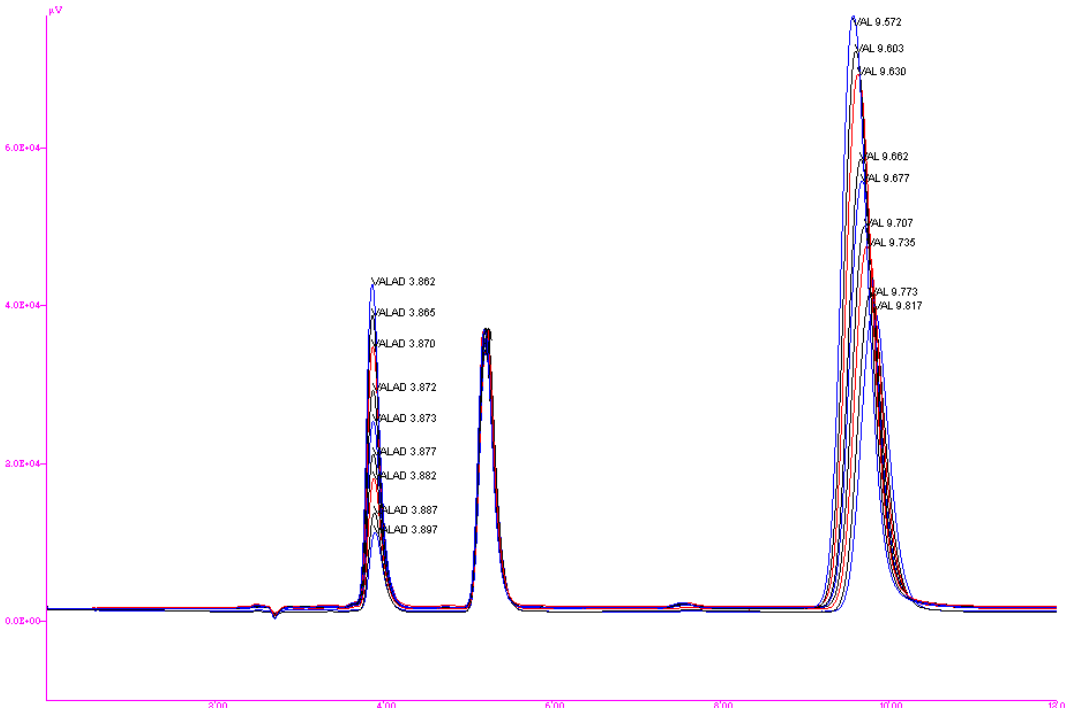

Figure 3. Chromatogram showing recovery study of valsartan in presence of acid degradant. VALAD (peak at extreme left): Degradant of valsartan by acid hydrolysis (RT3.86±0.04min); VAL (peak at extreme right):Valsartan (RT 9.57±0.08 min); IND (peak at middle): Indapamide as internal standard (RT 5.19±0.03 min).

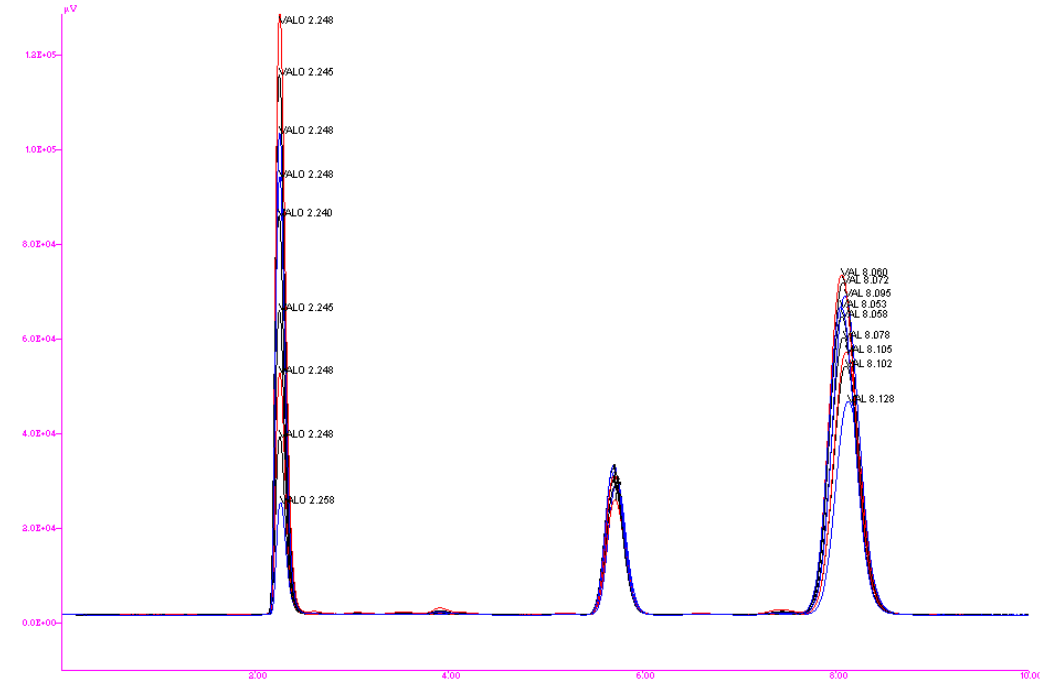

Figure 4. Chromatogram showing recovery study of valsartan in presence of oxidation degradant. VALO (peak at extreme left): Degradant of valsartan by oxidation (RT 2.24±0.04 min); VAL (peak at extreme right):Valsartan (RT 8.06±0.06 min); IND (peak at middle): Indapamide as internal standard (RT 5.70±0.03 min).

Table 6. System suitability parameters for acidic degradation of valsartan

Compound	R.T.	Theoretical plates	Selectivity	Capacity	Resolution	Asymmetry
VALAD	3.86	3542.39	0.00	385.17	0.00	1.40
VAL	9.57	4373.31	1.84	956.17	9.96	1.23
IND	5.19	4765.27	1.35	518.67	4.77	1.28

VALAD: Degradant of valsartan by acid hydrolysis; VAL: Valsartan; IND: Indapamide as internal standard

Table 7. System suitability parameters for oxidation degradation of valsartan

Compound	R.T.	Theoretical plates	Selectivity	Capacity	Resolution	Asymmetry
VALO	2.24	2720.83	0.00	223.83	0.00	1.46
VAL	8.06	3977.75	1.41	805.00	5.51	1.13
IND	5.70	4411.45	2.55	569.83	13.44	1.10

VALO: Degradant of valsartan by oxidation; VAL: Valsartan; IND: Indapamide as internal standard.

3.1. Degradation behaviour

In total, separately two major degradants were detected by HPLC on decomposition of the drug under acidic and oxidation conditions. The degradation behaviour of the drug in individual stress conditions is outlined below

3.1.1. Thermal stress

The exposure of the solid drug to 500C for 8 hrs did not result in significant decomposition. It indicated that VAL was stable to dry heat.

3.1.2. Hydrolysis

The drug degraded on heating at 700C for 1 hr in 1N HCl, forming major peak at RRT 0.40. The reaction in 0.1N NaOH at 700C for 1 hr did not result in significant degradation.

3.1.3. Oxidation

The drug was stable to 3% hydrogen peroxide at room temperature and no significant degradation was observed. However, decomposition occurred in 30% hydrogen peroxide, resulting in products resolving again at RRT 0.27.

3.1.4. UV degradation

The HPLC profile of light exposed drug sample was similar to those in the dark, indicating that light had no particular influence on the drug.

3.2. Development and optimization of the stability-indicating method

The resolution of degradants from the drug was influenced by concentration of methanol in the mobile phase. The acceptable separations with reasonable peak shapes and peak purity were achieved by using mobile phase comprising of methanol:water [70:30v/v, (pH 7.2, adjusted by adding 0.2 M ortho phosphoric acid into 0.2 M triethyl amine)] for acid hydrolysis stability-indicating assay. Also acceptable separations with reasonable peak shapes and peak purity were achieved using mobile phase methanol:water [60:40v/v, (pH 7.2, adjusted by adding 0.2 M ortho phosphoric acid into 0.2 M

triethyl amine)] for oxidation stability-indicating assay of VAL. The flow rate, injection volume and detection wavelength were adjusted as 1.2 ml/min, 20 µl and 250 nm, respectively in both assays.

3.3. Validation of the developed stability-indicating method

The validation studies established a linear response of the drug at concentrations between 5-45 µg/ml in mobile phase methanol:water (70:30, pH7.2). The mean values of slope, intercept, and coefficient of determination were shown in Table II A. Also the validation studies established a linear response of the drug at concentrations between 20-100 µg/ml in mobile phase methanol:water (60:40, pH7.2). The mean values of slope, intercept, and correlation coefficient were shown in Table II B. The

mean values for intra and inter-day precision were shown in Table III A. Also, the mean values for intra and inter-day precision were shown in Table III B.

3.4 Applicability of the developed method to stability samples

The developed method was found to apply even to real stability samples, which was verified through successful analyses of VAL, which had been stored at accelerated conditions of temperature (400C) and humidity (75% RH) for 3 months. The calibration curve shows good linearity. The method was validated according to prescribed ICH guidelines. The representative chromatogram was shown in Figure 5.

3.5 Characterization of degradation products

Mass chromatograms in the negative electron spray ionization (ESI) mode for the degradants are shown in Figure 6 and Figure 8. The *m/z* values of the peaks are 307.40 and 335.41 which matched 2-methyl-N-{[2'-(1H-tetrazol-5-yl)biphenyl-3-yl]methyl}propan-1-amine and N-methyl-N-{[2'-(1H-tetrazol-5-yl)biphenyl-3-yl]methyl}butanamide as degradant of VAL by acid hydrolysis and degradant of VAL by oxidation, respectively. The observed fragmentation pattern was taken into consideration for confirmation of chemical structures (Figure 7 and Figure 9)

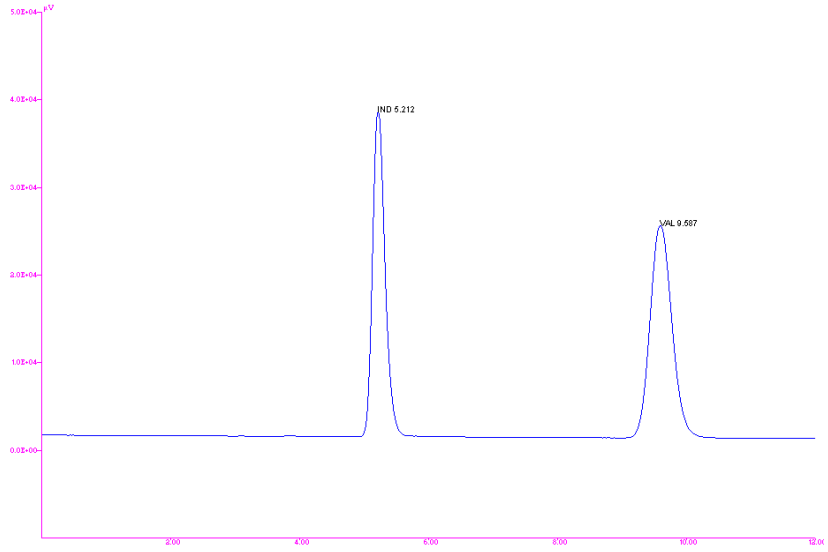

Figure 5. Representative chromatogram showing separation of drug in stability sample
VAL: Valsartan; IND: Indapamide as internal standard

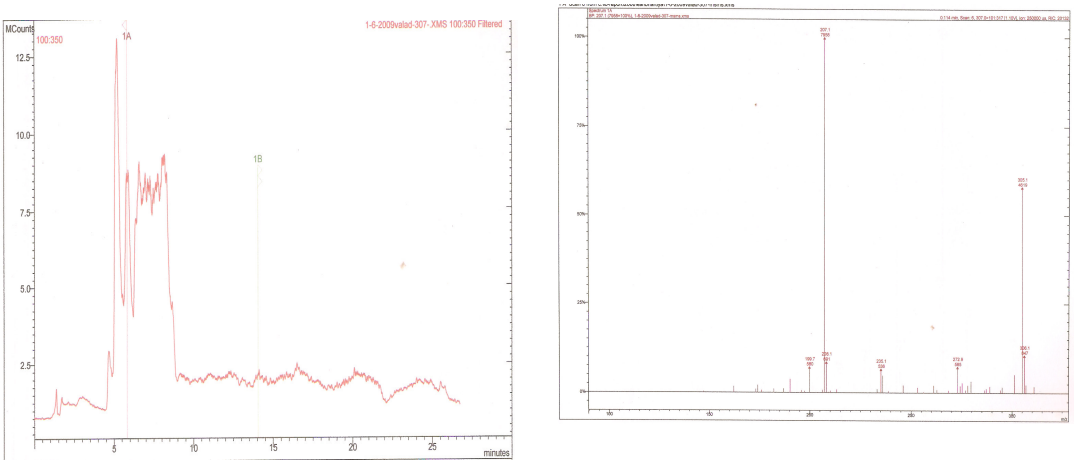

Figure 6. LC chromatogram and MS-MS spectra showing VALAD

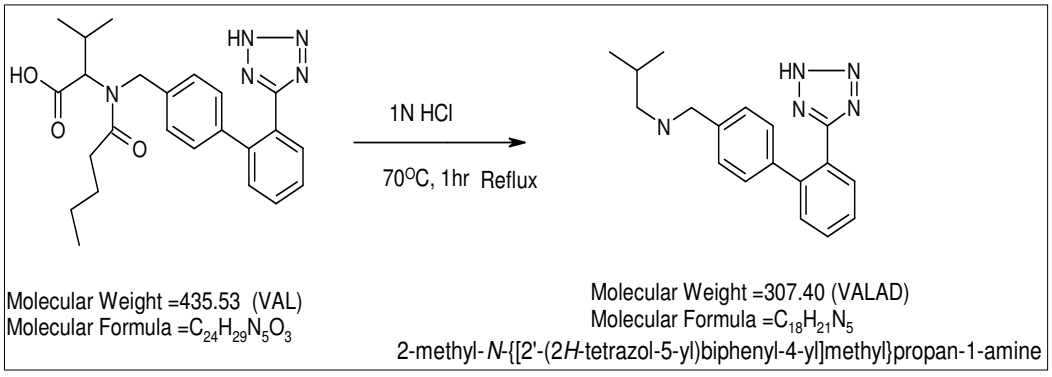

Figure 7. Structure of major degradation product (2-methyl-N-{[2'-(1H-tetrazol-5- yl)biphenyl-3-yl]methyl}propan-1-amine) of valsartan by acid hydrolysis

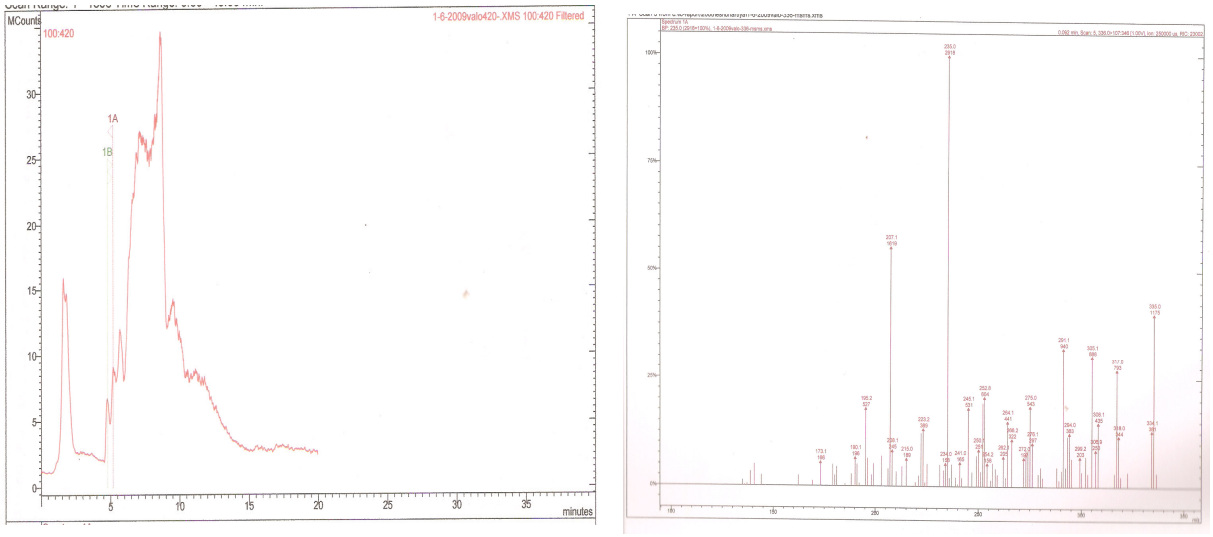

Figure 8. LC chromatogram and MS-MS spectra showing VALO

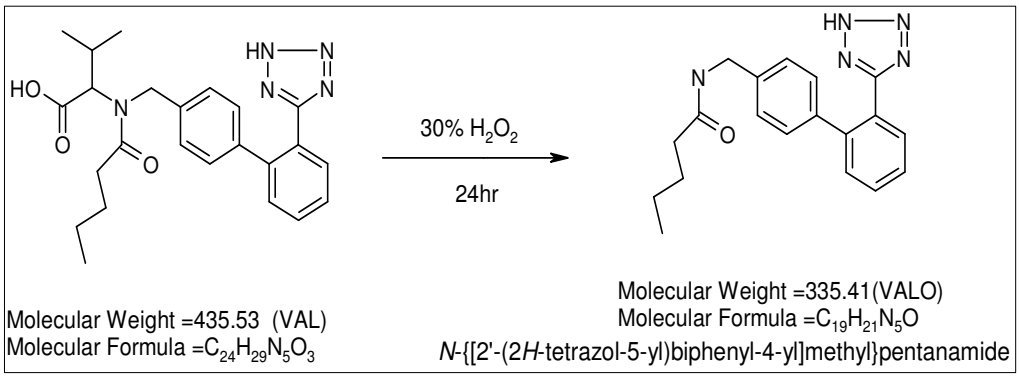

Figure 9. Structure of major degradation product N-methyl-N-{[2'-(1H-tetrazol-5- yl) biphenyl-3-yl] methyl} phenamide) of valsartan by oxidation

4. Conclusions

It was possible in this study to develop a stability-indicating HPLC assay method for VAL by subjecting the drug to ICH recommended stress conditions. The drug and degradants got well

separated from each other in an isocratic mode using a reversed-phase C18 column in mobile phase comprising of methanol:water [70:30v/v, (pH 7.2, adjusted by adding 0.2 M ortho phosphoric acid into 0.2 M triethyl amine)] which was used in acid hydrolysis stability-indicating assay and the mobile phase comprising of methanol:water [60:40v/v, (pH 7.2, adjusted by adding 0.2 M ortho phosphoric acid into 0.2 M triethyl amine)] in oxidation stability-indicating assay of VAL. The flow rate and detection wavelength were 1.2 ml/min and 250 nm, respectively. The method proved to be simple, accurate, precise, specific and selective. It was easily transferable to LC-MS. Also, it could be successfully employed for analysis of the drug and degradants in the marketed products stored for 3 months under accelerated conditions of temperature and humidity. The stress studies and subsequent LC-MS analyses showed that the drug was decomposed to degradation products, viz., 2-methyl-N-{[2'-(1H-tetrazol-5-yl)biphenyl-3-yl]methyl}propan-1-amine and N-methyl-N-{[2'-(1H-tetrazol-5-yl)biphenyl-3-yl]methyl}butanamide as acid hydrolysis of VAL and oxidation of VAL, respectively. The degradation products were characterized through spectral studies. It may be pertinent to add here that this study can be extended to isolation and characterization of individual degradants.

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