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Stability-indicating UPLC method for determination of Valsartan and their degradation products in active pharmaceutical ingredient and pharmaceutical dosage forms

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ABSTRACT

A simple, precise, accurate stability-indicating gradient reverse phase ultra-performance liquid chromatographic (RP-UPLC) method was developed for the quantitative determination of purity of Valsartan drug substance and drug products in bulk samples and pharmaceutical dosage forms in the presence of its impurities and degradation products. The method was developed using Waters Aquity BEH C18 (100 mm × 2.1 mm, 1.7 μm) column with mobile phase containing a gradient mixture of solvents A and B. The eluted compounds were monitored at 225 nm, the run time was within 9.5 min, which Valsartan and its seven impurities were well separated. Valsartan was subjected to the stress conditions of oxidative, acid, base, hydrolytic, thermal and photolytic degradation. Valsartan was found to degrade significantly in acid and oxidative stress conditions and stable in base, hydrolytic and photolytic degradation conditions. The degradation products were well resolved from main peak and its impurities, proving the stability-indicating power of the method. The developed method was validated as per international conference on harmonization (ICH) guidelines with respect to specificity, linearity, limit of detection, limit of quantification, accuracy, precision and robustness. This method was also suitable for the assay determination of Valsartan in pharmaceutical dosage forms.

1. Introduction

Though high-performance liquid chromatography (HPLC) is a well-established reliable technique used in controlling the quality and consistency of active pharmaceutical ingredients (APIs) and dosage forms, it is often a slow technique because of the complexity of some of the samples, it could still be improved.

Ultra-performance liquid chromatography (UPLC) is a new category of separation technique based upon well-established principles of liquid chromatography, which utilizes sub-2 μm particles for stationary phase. These particles operate at elevated mobile phase linear velocities to affect dramatic increase in resolution, sensitivity and speed of analysis. Owing to its speed and sensitivity, this technique is gaining considerable attention in recent years for pharmaceutical and biomedical analysis. In the present work, this technology has been applied to the method development and validation study of related substance and assay determination of Valsartan bulk drug and dosage forms.

The chemical formula of Valsartan is *N*-(1-oxopentyl)-*N*-[[2-(1H-tetrazol-5-yl) [1,1-biphenyl]-4-yl]methyl]-L-valine (Fig. 1A). Valsartan is a potent, highly selective, and orally active antagonist at the angiotensin II AT1-receptor that is used for the treatment of hypertension. Very few methods appeared in the literature for the determination of VAL individually based on high-performance liquid chromatography (HPLC) [1–3]. Sampath et al. [4] described identification and characterization of potential impurities of Valsartan AT1 receptor antagonist. There has been some of estimation of assays of analyte in human plasma including the use of liquid chromatography [5–8] and some combination with other drugs using high pressure liquid chromatography and derivative spectroscopy [9–14].

The European Pharmacopoeia (Ph.Eur.) and United States Pharmacopoeia (USP) monograph methods for Valsartan related compounds cannot separate all the potential impurities and degradation compounds of Valsartan. However, the Ph.Eur. and USP monograph methods can resolve Imp-C and Imp-F related compounds of Valsartan and total run time is about 40 min. To the best of our knowledge, none of the currently available analytical methods (including the Ph.Eur. and USP method) can separate and

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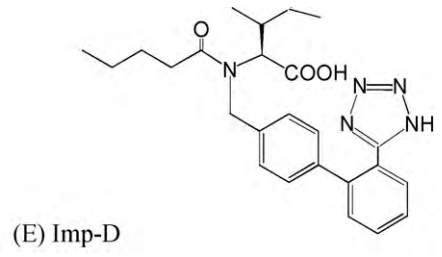
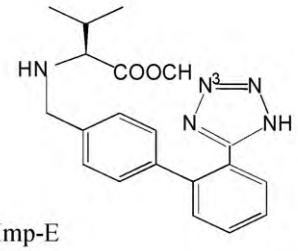
quantify all the known related compounds and degradation impurities of Valsartan API and dosage forms. Furthermore, there is no stability-indicating HPLC/UPLC method reported in the literature that can adequately separate and accurately quantify Valsartan API and dosage forms. It is, therefore, felt necessary to develop a new stability-indicating method for the related substance determination and quantitative estimation of Valsartan. We intend to opt for a faster chromatographic technique UPLC, for the said study. An attempt was made to determine whether UPLC can reduce analysis times without compromising the resolution and sensitivity.

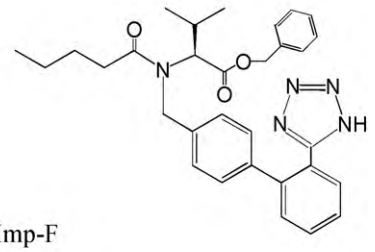
Hence a reproducible stability-indicating RP-UPLC method was developed for the quantitative determination of Valsartan and its seven impurities namely Imp-A, B, C, D, E, F and G (Fig. 1B–H). This method was successfully validated according to the International Conference Harmonization (ICH) guidelines (Validation of Analytical Procedures: Test and Methodology Q2). The method was also applied for study of in vitro dissolution profiles in pharmaceutical dosage forms.



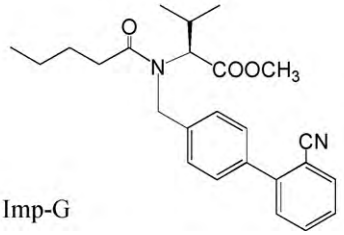
(E) Imp-D



(F) Imp-E

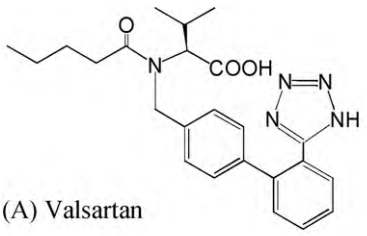


(G) Imp-F

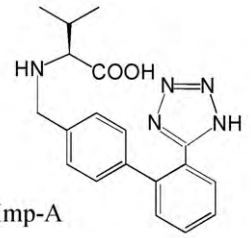


(H) Imp-G

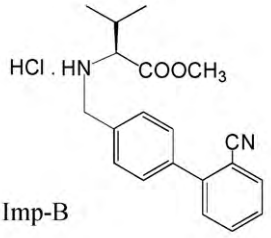
Fig. 1. Structures of Valsartan and its seven impurities. (A) Valsartan; (B) Imp-A; (C) Imp-B; (D) Imp-C; (E) Imp-D; (F) Imp-E; (G) Imp-F; and (H) Imp-G.



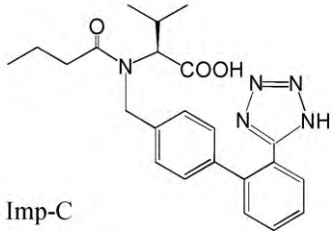
(A) Valsartan



(B) Imp-A



(C) Imp-B



(D) Imp-C

Fig. 1. *Continued.*

2. Experimental

2.1. Materials and reagents

Active pharmaceutical ingredient standards and samples were supplied by Dr. Reddy's Laboratories Limited, IPDO, Hyderabad, India. Commercially available Diovan tablets Novartis Pharmaceuticals Corporation, USA, were used for the dosage form analysis. The HPLC grade acetonitrile and analytical grade ortho phosphoric acid were purchased from Merck, Darmstadt, Germany. Water was prepared using Millipore Milli.Q Plus water purification system, Bedford, MA, USA.

2.2. Chromatographic conditions and equipments

LC was carried out on a Waters Aquity UPLC with photodiode array detector. The output signal was monitored and processed using empowers software. The chromatographic column used was acquity UPLC BEH C-18 100 mm, 2.1 mm, and 1.7 μm particle size. The separation was achieved on a gradient method. The solvent A contains a mixture of 1.0% acetic acid buffer, Acetonitrile in the ratio 90:10 (v/v); and the solvent B contains a mixture of 1.0% acetic acid buffer and acetonitrile in the ratio 10:90 (v/v), respectively.

The flow rate of mobile phase was 0.3 ml/min. The UPLC gradient program (*T*/%*B*) was set as 0.01/20, 1.0/40, 3.5/55, 6.5/80, 8.5/80, 8.9/20 and 9.5/20. The column temperature was maintained at 27 °C and the detection was monitored at a wavelength 225 nm. The injection volume was 1.0 μL.

2.3. LC–MS/MS conditions

LC–MS/MS system (Agilent 1200 series liquid chromatograph coupled with Applied Biosystems 4000 Q Trap triple quadruple mass spectrometer with Analyst 1.4 software, MDS SCIEX, USA) was used for the unknown compounds formed during forced degradation studies. Kromosil C18, 250 mm × 4.6 mm, 5 μm column (Grace Division Discovery Science, Deerfield, IL, USA) was used as stationary phase. 1.0% acetic acid (Merck, Darmstadt, Germany) was used as buffer. Buffer and acetonitrile in the ratio 90:10 (v/v); was used as solvent A and buffer and acetonitrile in the ratio 10:90 (v/v); was used as solvent B. The gradient program (*T*/%*B*) was set as 0.01/20, 5/20, 20/40, 30/55, 40/80, 50/80, 52/20 and 60/20. Solvent A was used as diluent. The flow rate was 1.0 ml/min. The analysis was performed in positive electro spray positive ionization mode. Ion Source voltage was 5000 V. Source temperature was 450 °C. GS1 and GS2 are optimized to 30 and 35 psi, respectively. Curtain gas flow was 20 psi.

2.4. Preparation of stock solutions

A stock solution of Valsartan (50 μg/ml) was prepared by dissolving an appropriate amount of drug in acetonitrile: water 20:80 (v/v), respectively. Working solutions containing 50 and 5 μg/ml were prepared from this stock solution for determination of related substances and for assay determination, respectively. A mixed stock solution (0.5 μg/ml) of the impurities (denoted Imp-A to Imp-G) was also prepared in diluent.

2.5. Preparation of sample solution

Twenty ($n = 20$) Valsartan 160 mg Diovan tablets were weighed and the pellets were transferred in to a clean, dry mortar. Pellets equivalent to 50 mg drug were dissolved in 100 ml of methanol to give a solution containing 0.5 mg/ml. 1.0 ml of this solution was diluted to 10 ml with acetonitirle:water 20:80 (v/v), to give a solution containing 50 μg/ml and filtered through a 0.22-μm Nylon membrane filter. The solution obtained was analyzed by UPLC.

2.6. Stress studies

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities [15]. The specificity of the developed LC method for Valsartan was carried out in the presence of its seven impurities. Stress studies were performed at an initial concentration 500 μg/ml of Valsartan on tablets to provide an indication of the stability-indicating property and specificity of the proposed method. Intentional degradation was attempted to stress condition of UV light (254 nm), heat (60 °C), acid (2N HCl at 60 °C), base (0.05N NaOH at 60 °C), hydrolytic (60 °C) and oxidation (6.0% H_2O_2 at 60 °C) to evaluate the ability of the proposed method to separate Valsartan from its degradation products. For heat and light studies, study period was 10 days whereas for hydrolytic, base it was 24 h; acid 1 h and oxidation it was 5 h.

The purity of peaks obtained from stressed samples was checked by use of the PDA detector. The purity angle was within the purity threshold limit obtained in all stressed samples and demonstrates the analyte peak homogeneity. Assay of stressed samples was performed by comparison with reference standards and the mass balance (% assay + % impurities + % degradation products) for stressed samples was calculated. Assay was also calculated for Valsartan sample by spiking all five impurities at the specification level (i.e. 0.15%).

3. Method validation

The described method has been validated for assay and related substances by UPLC determination [16].

3.1. Precision

The repeatability of the related-substance method was checked by sixfold analysis of 50 μg/ml Valsartan spiked with 0.15% of each of the seven impurities. The RSD (%) of peak area was calculated for each impurity.

Inter- and intra-day variation and analyst variation was studied to determine intermediate precision of the proposed method. Intra-day precision was determined by sixfold analysis of 50 μg/ml Valsartan spiked with 0.15% of each of the seven impurities. The same protocol was followed for three different days to study inter-day variation ($n = 18$). Different analysts prepared different solutions on different days. The RSD (%) of peak area was calculated for each impurity.

The precision of the assay was evaluated by performing six independent assays of a test sample of Valsartan and comparison with a reference standard. The RSD (%) of the six results was calculated.

3.2. Limit of detection (LOD) and quantification (LOQ)

The LOD and LOQ for Valsartan and its impurities were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. Precision study was also carried out at the LOQ level by injecting six ($n = 6$) individual preparations and calculating the % RSD of the area for each impurity and for Valsartan.

3.3. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the true value and the value found. The accuracy of the assay method for Valsartan was evaluated in triplicate at three concentrations, 2.5, 5.0 and 7.5 μg/ml on drug product and recovery was calculated for each added concentration.

For impurities, recovery was determined in triplicate for 0.075%, 0.15%, and 0.225% of the analyte concentration (50.0 μg/ml) on drug product and recovery of the impurities was calculated.

3.4. Linearity of response

Detector response linearity for all seven and Valsartan was assessed by injecting eight separately prepared solutions covering the range LOQ to 200% (LOQ, 0.015%, 0.0375%, 0.075%, 0.1125%, 0.15%, 0.1875%, 0.225%, 0.2625% and 0.30%) of the normal sample concentration (50.0 μg/ml). The correlation coefficients, slopes and *Y*-intercepts of the calibration curve were determined.

3.5. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

To determine the robustness of the method the experimental conditions were deliberately changed. The resolution of Valsartan and the seven impurities were evaluated. The mobile phase flow rate was 0.30 ml/min; to study the effect of flow rate on

resolution it was changed to 0.27 and 0.33 ml/min. The effect of column temperature was studied at 22 °C and 35 °C (instead of 27 °C).

3.6. Solution stability and mobile phase stability

Valsartan solutions (spiked) prepared in diluent were injected at 0 h, 24 h and 48 h of time intervals, calculated the impurity content (Imp-A to Imp-G impurity) and checked the consistency in the % area of the principal peak at each interval. Mobile phase prepared was kept constant during the study period.

The mobile phase study was demonstrated by injecting the freshly prepared sample solution at different time intervals (0 h, 24 h and 48 h).

4. Results and discussion

4.1. Method development and optimization

The main objective of the chromatographic method was to separate critical closely eluting pair of compounds Valsartan and Imp-D; Imp-A and Imp-B; Imp-G and Imp-F and to elute Valsartan as a symmetrical peak. The blend containing 50 μg/ml of Valsartan and 2 μg/ml of each impurity (seven) in the solvent A. Initially, experiments were carried out based on the available methods as per USP/EP monograph, but the method specified in the official monographs does not have an adequate separation of Imp-A and Imp-B, highly retained of Imp-G. Moreover the method requires longer run time of around 70 min. Considering the demerits of the existing methods, felt necessary to develop a new stability-indicating method for the related substance and assay of pharmaceutical dosage forms determination. We intend to opt for a faster chromatographic technique UPLC, for the said study. An attempt was made to determine whether UPLC can reduce analysis times without compromising the resolution (Rs) and sensitivity.

Valsartan spiked solutions were subjected to separation by reversed-phase LC on a Waters Acquity BEH C18, 50 mm × 2.1 mm, 1.7 μm column with 1.0% acetic acid buffer, acetonitrile in the ratio of 50:50 (v/v) with isocratic flow. Two compounds viz., Imp-A and Imp-B were merged together, Imp-D was co-eluted with Valsartan and also Imp-G was strongly retained. With changing of mobile phase composition buffer, acetonitrile 60:40 and 40:60 (v/v), three compounds viz., Imp-A and Imp-B did not separate well but still Imp-G was strongly retained. Attempts were made with gradient elution with solvent A and B using different C18 UPLC columns (Inertsil ODS-3, 50 mm × 2.1 mm, 2 μm particles and Zorbax XDB C-18, 50 mm × 4.6 mm, containing 1.8 μm particles, Phenyl 100 mm × 2.1 mm, 1.7 μm), and using gradient elution with solvent A and B. But at all above columns and intended experimental conditions, separation of impurities was not satisfactory. It was found that use of 1.0% acetic acid buffer, acetonitrile in the ratio of 90:10 (v/v) as solvent A; 1.0% acetic acid buffer, acetonitrile in the ratio of 10:90 (v/v) was solvent B; with gradient elution (*T*/*%B*) was set as 0.01/20, 1.0/40, 3.5/55, 6.5/80, 8.5/80, 8.9/20 and 9.5/20 gives enabled separation for all pair of compounds and eluted Valsartan as a symmetrical peak (Fig. 2A) and there was no interference of excipients (placebo) with impurity peaks and Valsartan analyte peak.

However verified other buffer solutions like phosphoric acid (1.0%), 0.01 mM sodium and potassium dihydrogen orthophosphates; column oven temperature is also studied at room temperature 27 °C and 40 °C; in view of possible interference study, attempts were also made by adjusting the mobile phase flow rate to separate the all process related impurities. But no significant impact is observed on the critical chromatographic parameters (resolution, USP tailing and retention time). After several preliminary investigatory chromatographic runs, on the basis of suitability (mass compatibility) for drug content estimation and cost, because of rapid and economic analysis is becoming increasingly important in pharmaceutical analysis to increase throughput, under the final stated experimental conditions described.

System suitability parameters were evaluated for Valsartan and its seven impurities. Tailing factor for all seven impurities and Valsartan was found <1.2. USP Resolution of Valsartan and seven potential impurities were >2.0 for all pairs of compounds.

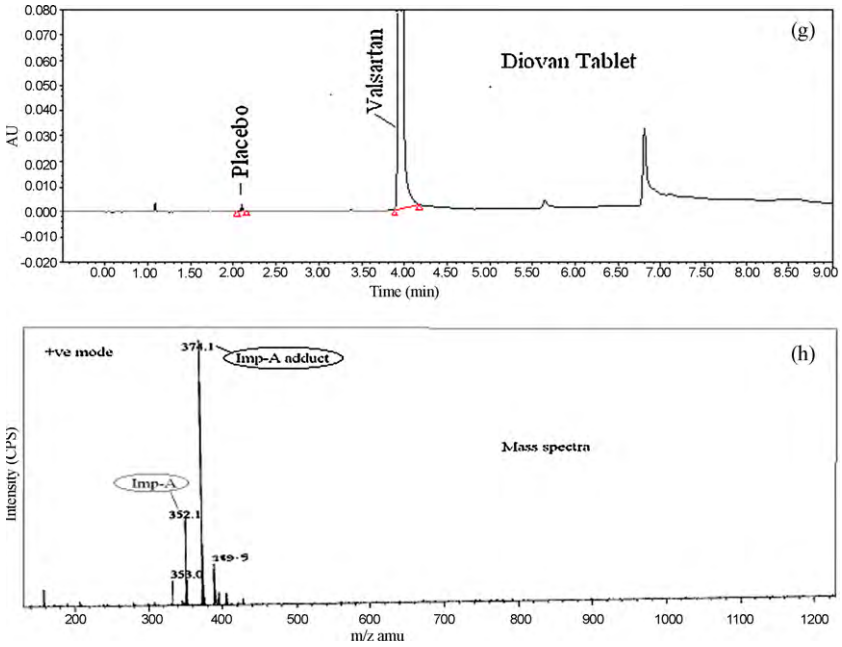


Fig. 2. Blend chromatogram of Valsartan and its impurities. (A) Valsartan spiked chromatogram; (B) Valsartan purity plot; (C) base degradation chromatogram; (D) water hydrolysis degradation chromatogram; (E) oxidative degradation chromatogram; (F) acid degradation chromatogram; (G) tablet chromatogram; and (H) mass spectra.

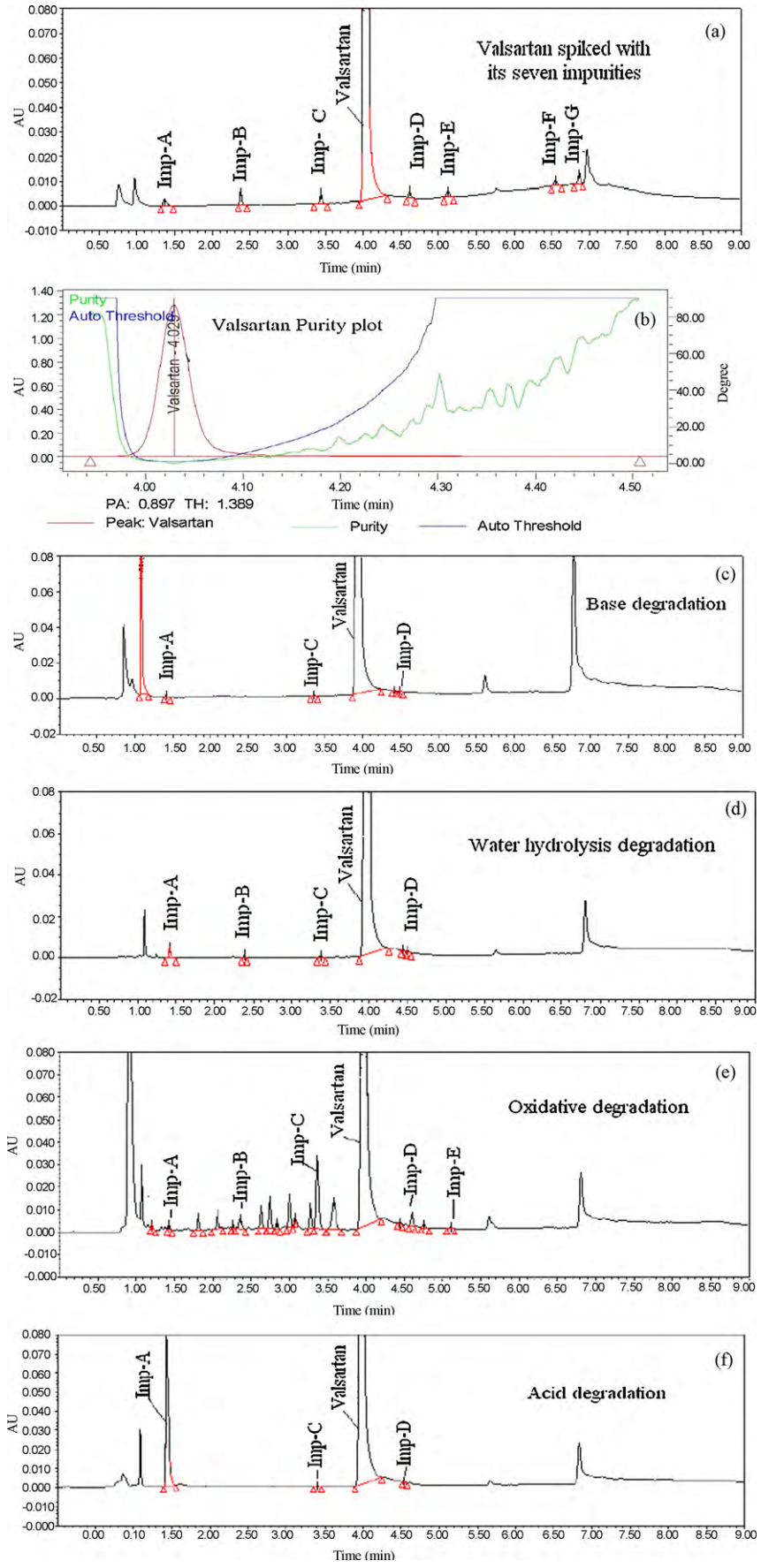


Fig. 2. (*Continued*).

Table 1
LOD, LOQ, regression and precision data.

Parameter	Valsartan	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	Imp-F	Imp-G
LOQ (μg/ml)	0.007	0.005	0.00022	0.007	0.007	0.006	0.006	0.006
LOD (μg/ml)	0.0020	0.0015	0.00065	0.0019	0.0022	0.0018	0.0019	0.0017
Regression equation (y)								
Slope (b)	602,251	579,659	630,092	652,924	646,925	633,737	614,116	657,306
Intercept (a)	207	220	403	115	65	8.1	156	−135
Correlation coefficient	0.9979	0.9995	0.9994	0.9996	0.9999	0.9999	0.9992	0.9987
Y-intercept at 100% level	3.1%	2.4%	4.0%	1.7%	1.0%	0.1%	2.5%	−2.1%
R square value	0.9958	0.9988	0.999	0.9992	0.9997	0.9997	0.9984	0.9974
Precision (% RSD)[a]	0.1	2.2	1.2	1.9	1.9	2.8	2.9	0.1
Intermediate precision (% RSD)[a]	0.2	1.2	4.6	0.9	1.3	2.5	2.0	1.4

Linearity range is LOQ to 150% with respect to 50 μg/ml Valsartan for impurities.
[a] Six determinations using LOQ solution for impurities.

Table 2
Summary of forced degradation results.

Degradation condition	Time	Assay (% (w/w) on anhydrous basis)	Mass balance (% assay + deg. products)	RS by UPLC % degradation	Remarks/observation
HCl, 2N 60 °C (acid hydrolysis)	1 h	87.2%	99.8	12.6%	Impurity-A degradation product were formed
NaOH, 0.05N 60 °C (base hydrolysis)	24 h	99.3%	99.9	0.3%	No significant degradation observed
Water hydrolysis (60 °C)	24 h	99.6%	99.8	0.2%	No significant degradation observed
Oxidation by H_2O_2, 6.0%, 60 °C	5 h	88.0%	99.4	11.4%	Impurity-B, C and D degradation products were formed
Thermal (60 °C) solid	10 days	99.7%	99.9	0.2%	No degradation observed
UV at 254 nm	10 days	99.8%	99.9	0.2%	No degradation observed

4.2. Validation of the method

4.2.1. Precision

RSD (%) in the study of the repeatability of the assay of Valsartan was within 1.1%. The % RSD of peak area for the seven impurities namely Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G in the study of the repeatability was in Table 1. RSD (%) results of Valsartan and its impurities for intermediate precision (intra- and inter-day repeatability) are within 5.0%. These results confirmed that the method was highly precise.

4.2.2. Limits of detection and quantification

The determined limit of detection, limit of quantification and precision at LOQ values for Valsartan and its seven impurities are reported in Table 1.

4.2.3. Accuracy

Recovery of Valsartan from pharmaceutical dosage forms ranged from 98.3% to 101.5%. Recovery of the seven impurities from pharmaceutical dosage forms ranged from 93.6% to 106.8%.

4.2.4. Linearity

For all seven impurities and Valsartan, linear calibration curve was obtained ranging from LOQ to 0.3% (LOQ, 25%, 50%, 75%, 100%, 125%, 150% and 200%). The correlation coefficient obtained was >0.9979 in both cases (Table 1). Linearity was determined over three consecutive days. The RSD values of the slope and Y-intercept values were 1.3% and 5.2%, respectively, which confirmed the linear relationship between peak areas and concentrations. The results indicate very good linearity.

4.2.5. Robustness

In all the deliberate varied chromatographic conditions (flow rate and column temperature), all analyte peaks were adequately resolved and elution orders remained unchanged. Resolution between Imp-D and Valsartan was >2.0 and resolution between Imp-A and Imp-B was >2.5 for all flow rate (0.27 and 0.33 ml/min) and column temperature (22 °C and 33 °C) variation conditions.

4.2.6. Stability in solution and in the mobile phase

RSD (%) for assay of Valsartan during solution stability and mobile phase stability experiments was within 1.2%. No significant changes in the amounts of the seven impurities were observed during solution stability and mobile phase experiments when performed using the related substances method. The results from solution stability and mobile phase stability experiments confirmed that standard solutions and solutions in the mobile phase were stable for up to 48 h during assay and determination of related substances.

4.2.7. Results from forced degradation studies

All forced degradation samples were analyzed at an initial concentration 50 μg/ml of Valsartan with UPLC conditions mentioned in conditions using PDA detector to ensure the homogeneity and purity of Valsartan peak (Fig. 2B). Degradation was not observed when Valsartan was subjected to hydrolytic, base, light and heat conditions. Significant degradation was observed when the drug was subjected to oxidative hydrolysis (6.0% H_2O_2 at 60 °C for 5 h) and acid (2N HCl at 60 °C for 1 h) leading to the formation of Imp-A (Fig. 2C–G). This was confirmed by co-injecting Imp-A standard to these degraded samples and by LC–MS/MS analysis. LC–MS/MS analysis was performed as per experimental conditions and mass of the impurity was 351 which was corresponding to the mass of Imp-A (Fig. 2H). Results from force degradation studies were presented in Table 2.

Assay studies were carried out for stress samples (at 5 μg/ml) against Valsartan qualified reference standard. The mass balance (% assay + % sum of all compounds + % sum of all degradants) results were calculated for all stressed samples and found to be more than 99%. The purity and assay of Valsartan was unaffected by the pres-

ence of its impurities and degradation products and thus confirms the stability-indicating power of the developed method.

5. Conclusion

The rapid gradient RP-UPLC method developed for quantitative analysis of Valsartan and related substances in pharmaceutical dosage forms is precise, accurate, linear, robust and specific. Satisfactory results were obtained from validation of the method. The retention time (9.5 min) enables rapid determination of the drug. This method exhibited an excellent performance in terms of sensitivity and speed. The method is stability-indicating and can be used for routine analysis of production samples and to check the stability of samples, it may be useful for study of in vitro dissolutions pharmaceutical dosage forms and to check the stability of samples of Valsartan.

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