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UPLC Method for Simultaneous Determination of Valsartan & Hydrochlorothiazide in Drug Products

Antil P[1], Kaushik D[1], Jain G[2], Srinivas KS[2] and Indu Thakur[3]*

[1]*Rajendra Institute of Technology and Sciences, Hisar Road, Sirsa125055, Haryana, India*
[2]*Daiichi Sankyo Research Centre in India, Sector 18, Gurgaon 122015, Haryana, India*
[3]*Indian Institute of Technology, BHU, India*

Abstract

A new, simple, precise and stability-indicating UPLC (Ultra Performance Liquid Chromatography) method was developed and validated for the simultaneous determination of anti-hypertensive drug Valsartan (VAL) and Hydrochlorothiazide (HCTZ) in combined dosage forms. The method was developed using Kromasil eternity C-18 column (50 mm×2.1 mm, 3.5 µm) with isocratic elution. Triethylamine buffer (0.1% v/v) and methanol (75:25 v/v) were used as mobile phase with 0.6 mL min^{-1} flow rate at room temperature. The detection wavelength was fixed at 225 nm; the run time was within 2 min. The method was validated in terms of linearity, accuracy and reproducibility. Calibration plots were linear over the range of 7-13 µg/ml for HCTZ and 56-104 µg/ml for VAL. Recovery was in the range of 98-102% with the relative standard deviation of less than 2% for both drugs. The limit of detection and the limit of quantification for the valsartan were found to be 0.8 and 2.4 µg/ml respectively and for hydrochlorothiazide 0.12 and 0.36 µg/ml respectively. The proposed method was also suitable for determination of VAL & HCTZ in bulk and pharmaceutical dosage forms.

Keywords: Valsartan; Hydrochlorothiazide; UPLC

Introduction

Ultra Performance Liquid Chromatography (UPLC) [1] system is an innovative product that brought revolution in high performance liquid chromatography by outperforming conventional high performance liquid chromatography (HPLC). UPLC decreases sample run times up to a factor of 10, uses up to 95 percent less solvent and significantly improves productivity in the laboratory. UPLC achieves the speed by using novel sub two-micron particles that reduces chromatographic run times and improves resolution. UPLC was designed as a total system to leverage both ultra-high pressure and small particle separation attributes that result in uniquely superior performance with significant improvements in resolution, sensitivity and speed [2]. UPLC system eliminates significant time and cost per sample from analytical process while improving the quality of results, the system allows chromatographers to work at higher efficiencies, flow rates, and back pressures [3]. UPLC photodiode array (PDA) detector detects and quantifies lower concentrations of sample analyte, trace impurities with maximum sensitivity and compares spectra across wavelengths and broad concentration ranges [4]. The present study was conducted to separate and quantify valsartan and hydrochlorthiazide in combined tablet dosage form by using RP-UPLC technique.

Valsartan, (S)-N-(1-Oxopentyl)-N-[[2'-(1*H*-tetrazol-5- yl) [1,1'-biphenyl]-4-yl]methyl]-L-valine, is an orally active specific angiotensin II receptor blocker effective in lowering blood pressure in hypertensive patients. It is a selective type-1 angiotensin II receptor antagonist which blocks the blood pressure increasing effects of angiotensin II via rennin-angiotensin-aldosterone system. It is used as a first line agent to treat uncomplicated hypertension, isolated systolic hypertension and left ventricular hypertrophy. Very few methods appeared in the literature for the determination of VAL individually based on HPLC. There has been some estimation of assays of analyte in human plasma including the use of liquid chromatography and some combination with other drugs using highpressure liquid chromatography and derivative spectroscopy [5-9].

Hydrochlorothiazide (6-chloro-3,4-dihydro-2H- 1,2,4-benzothiadiazine- 7-sulfonamide-1,1- dioxide) is a diuretic of the class of benzothiadiazines widely used in antihypertensive pharmaceutical formulations, alone or in combination with other drugs, which decreases active sodium reabsorption and reduces peripheral vascular resistance. It was successfully used as one of the content in association with other drugs in the treatment of hypertension II. Numerous publications described the determination of HCTZ concentration in plasma or urine by HPLC with ultraviolet or electrochemical detection. Several chromatographic methods have been reported for the analysis of HCTZ individually or in combination, such methods have included: HPLC coupled with UV or diode array, electrochemical detection, LC–MS or with tandem LC–MS/MS [10-14]. There are a few HPLC methods appearing in the literature for the simultaneous determination of VAL and HCTZ in tablets, wherein longer run time (8 to 15 min) along with a complex mobile phase combinations has been used [13,14]. Since the available methods were based on HPLC, LC-MS, GC-MS, capillary electrophoresis [15] and UV-derivative spectrophotometry, the procedure was inconvenient for determination and the run times were rather long [16-19]. Simultaneous determination of both drugs is highly desirable as this would allow more efficient generation of clinical data and could be more cost-effective than separate assays. To the best of our knowledge, there are no reports available on RP-UPLC method for VAL & HCTZ in combined tablet dosage form with short run time. It is, therefore, felt necessary to develop a new method for simultaneous determination of both the drugs with shorter run time. We intend to opt for a faster chromatographic technique UPLC, for the said study.

***Corresponding author:** Indu Thakur, Indian Institute of Technology, BHU, India, E-mail: prasunantilvali@yahoo.com

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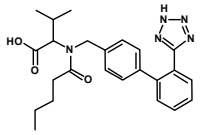
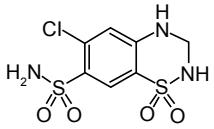
An attempt was made to determine whether UPLC can reduce analysis times without compromising the resolution and sensitivity.

Hence a rapid, simple and precise reverse-phase Ultra performance liquid chromatographic method was developed and validated for simultaneous estimation of valsartan and hydrochlorothiazide in tablet dosage form.



VALSARTAN



HYDROCHLORTHIAZIDE

Material and Method

Materials

The reference sample of valsartan and hydrochlorothiazide was supplied by Ranbaxy laboratory limited, gurgaon. Tablet for analysis were of Lupin pharmaceuticals available in the market under brand name of Valent-H having combination of valsartan and hydrochlorothiazide 80 mg and 12.5 mg respectively. HPLC grade methanol and acetonitrile were purchased from S.D. Fine Chemicals Ltd., Mumbai, orthophosphoric acid of AR Grade from Qualigens whereas triethylamine of HPLC grade was obtained from Thermo Fischer scientific Ltd. Reagents as ammonium acetate, isopropyl alcohol, hydrochloric acid, sodium hydroxide, hydrogen peroxide were of analytical grade obtained from merck (India). Water was collected from Milli Q purification unit with 0.22 µ filter.

Chromatographic conditions: The analysis of the drug was carried out on a Waters Acquity UPLC system (Milford, MA, USA) equipped with a binary solvent (loop capacity 10 µl), column manager composed of column oven, pre column heater, (50 mm×2.1 mm) column with 3.5 µ particle size and a photo diode array detector. Data acquisition, data handling and instrumentation control were performed by Empower software version 2.0. Kromasil C-18 column was used to optimize the method.

Sample preparation: Standard solution was prepared by dissolving HCTZ and VAL in 100 ml diluents (methanol: water, 80:20 v/v) to obtain a concentration of 12.5 µg/ml and 80 µg/ml respectively. Ten tablet of Valent-H containing VAL and HCTZ as active ingredient were weighed and finely powdered. Equivalent amount to standard APIs of powder was weighed, transferred in 1000 ml volumetric flask, diluted with methanol: water 80:20 v/v, sonicated for 30 min and then same diluent was added to make up the volume up to 1000 ml.

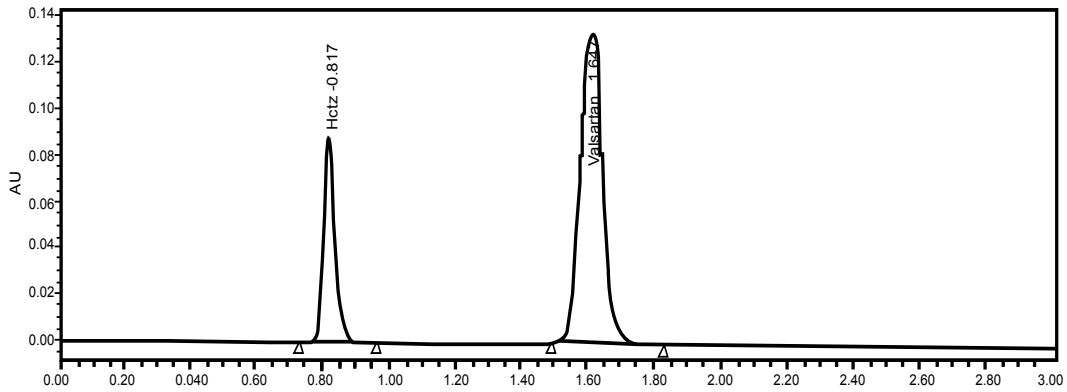


Figure 1: UPLC Chromatogram of HCTZ and VAL.

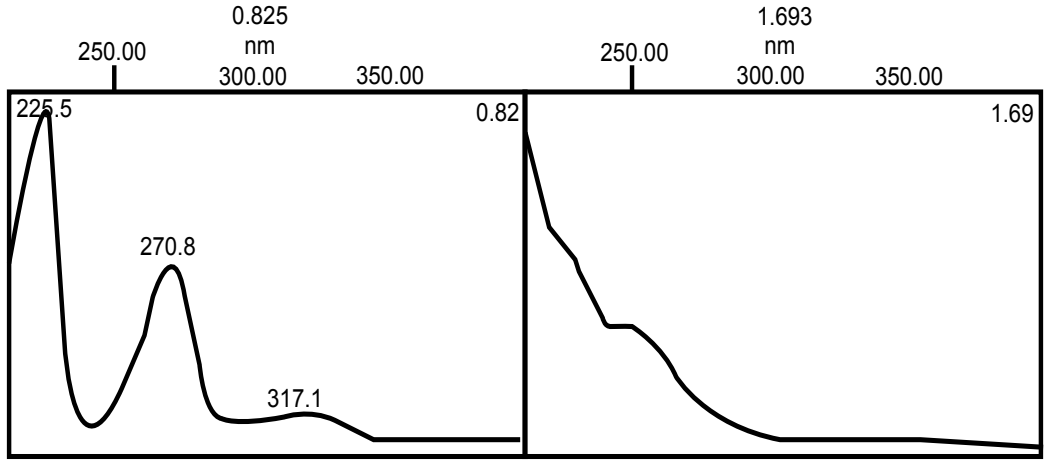


Figure 2: UV spectra of HCTZ and VAL respectively.

Method development

A variety of mobile phases were investigated in the development of a stability-indicating UPLC method for the analysis of HCTZ and VAL. A mixture of methanol and 0.1% v/v triethylamine (pH 3.0 adjusted with o-Phosphoric acid) (75:25, v/v) was found to be the most suitable mobile phase for ideal separation of HCTZ and VAL. The solvent mixture was filtered through a 0.22 µ PVDF filter and sonicated before use. It was pumped through the column at a flow rate of 0.6 ml/min. The column was maintained at an ambient temperature. The detection of the drug was monitored at 225 nm. The run time was set at 2 min. Under these optimized chromatographic conditions the retention time obtained for the drugs HCTZ and VAL was 0.817 min and 1.647 min respectively. A typical chromatogram showing the separation of the drugs is as shown in Figure 1.

Wavelength selection: The UV spectra of individual drug substances was recorded in methanol: water (80:20) solvent system as depicted in Figure 2. From the UV spectra wavelength 225 nm selected because at this combination both drugs contribute linear relationship between absorbance and concentration.

Validation of developed and optimized method: The validation of developed method was doneas per ICH guidelines [20,21] which include System suitability (retention time, peak area), Precision (system precision, method precision), Accuracy, Linearity, Robustness (flow rate, wavelength, mobile phaseratio, column temperature, pH) and Solution stability at 25°C.

Specificity: The specificity of the method was determined by checking theinterference of placebo with analyte and the proposed method was evaluated by checking peak purity, USP tailing, plate count and resolution of HCTZ & VAL during study. The peak purity of both the drugs was found satisfactory under different conditions as shown in Table 1.

Precision: The precision of the proposed method was checked by carrying out six independent assays of test samples. Mean, SD and an RSD (%) value of six assays was calculated. Intermediate precision was carried out by analyzing the samples on a different day on another instrument. System precision and method precision both were under the permissible limit i.e. 1% and 2% respectively (Table 2).

Injection	Name	Retention Time	Purity Angle	Purity threshold	Area	USP tailing	USP plate count	Resolution
Standard	HCTZ	0.824	0.125	1.101	308611	1.57	2467	
	VAL	1.681	0.08	1.039	866315	1.24	2763	6.96
Sample	HCTZ	0.823	0.169	1.094	307758	1.56	2452	
	VAL	1.681	0.055	1.035	866245	1.25	2757	6.96

Table 1: Specificity data for HCTZ and VAL.

	Repeatability		Intermediate Precision	
S. No.	HCTZ	VAL	HCTZ	VAL
1	95.66	100.04	97.65	99.71
2	95.68	100.04	97.49	102.19
3	95.46	99.23	97.69	102.16
4	95.6	99.81	97.66	102.11
5	95.53	99.48	97.51	102.11
6	95.68	99.86	97.76	102.12
Mean	95.6	99.74	96.61	100.74
SD	0.08	0.325	1.01	1.20
% RSD	0.09	0.33	1.01	1.19

Table 2: Precision data for HCTZ and VAL.

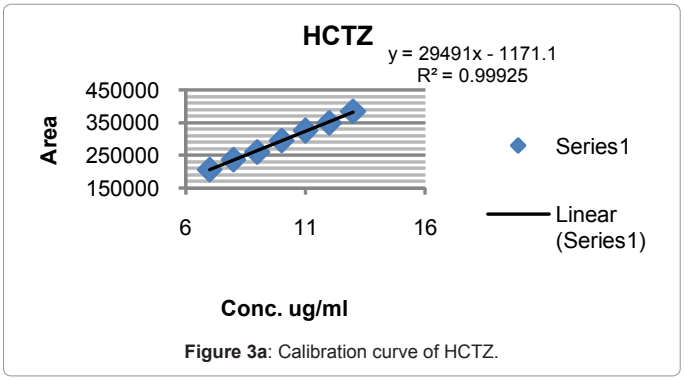


Figure 3a: Calibration curve of HCTZ.

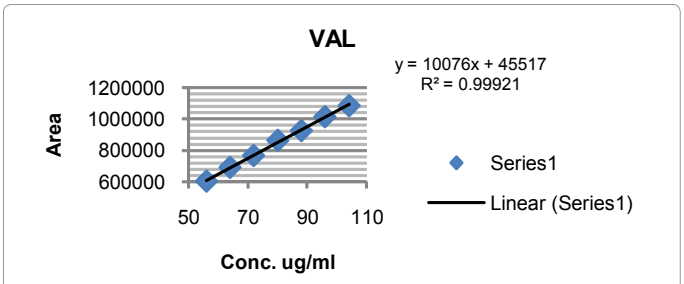


Figure 3b: Calibration curve of VAL.

Linearity: Standard stock solution of the drug was diluted to prepare linearity standard solutions in the concentration range of 70-130% of standard concentration. The calibration curve of analytical method was assessed by plotting concentration versus peak area and represented graphically in Figure 3a and 3b. It was showing suitable linearity in the range of 7-13 µg/ml for HCTZ and 56-104 µg/ml for VAL respectively.

Accuracy: To assess the accuracy both the samples were studied in three different concentrations of 80%, 100% & 120% and recovery was found within the acceptance criteria i.e. 95-105% as shown in Table 3.

LOD & LOQ: The limit of detection (LOD) & limit of quantitation (LOQ) was calculated on the basis of signal to noise ratio of 3:1 and 10:1 respectively. The lowest limit of quantitation for HCTZ and VAL was found to be 0.36 µg/ml & 2.4 µg/ml respectively. The lowest limit of detection for HCTZ and VAL was found to be 0.12 µg/ml & 0.8 µg/ml respectively. Chromatograms showing LOD & LOQ is as shown in Figure 4 and 5 respectively.

Robustness: The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (± 0.02 mL min^{-1}), wavelength (± 5 nm), column oven temperature (± 5.0°C), pH of buffer in mobile phase (± 0.2) and organic composition (± 2 from absolute value). % RSD for all the robustness studies is as shown in Table 4.

Solution stability: To assess the solution stability, standard and test solutions were kept at 25°C (laboratory temperature) for 24 hrs. They were analysed at 6 different time intervals. The cumulative RSD (%) at each time interval is shown in the Table 5 and 6.

Conclusion

The rapid isocratic RP-UPLC method developed for quantitative

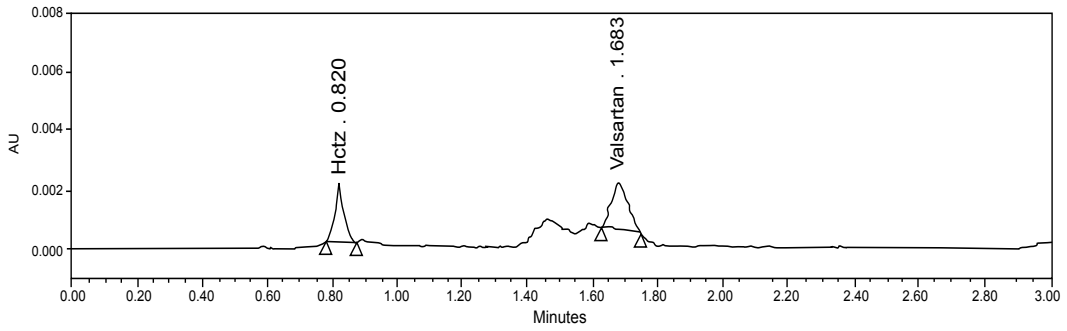


Figure 4: UPLC chromatogram for Limit of detection of HCTZ and VAL.

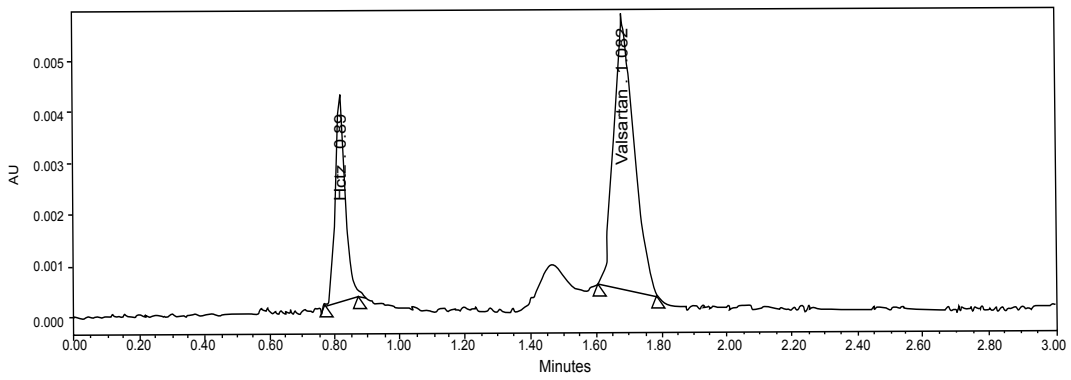


Figure 5: UPLC chromatogram for Limit of quantitation of HCTZ and VAL.

Parameter	HCTZ (7-13 ug/ml)	VAL (56-104 ug/ml)
Slope	29492	10076
Intercept	-1172	45517
Correlation coefficent	0.9992	0.9992

Table 3: Linearity data for HCTZ and VAL.

	%Recovery	
Concentration (%)	**HCTZ**	**VAL**
80	98.53	99.65
100	101.74	99.00
120	97.91	100.12

Table 4: Accuracy data for HCTZ and VAL.

	% RSD	
Robustness parameters	**HCTZ**	**VAL**
flow rate (± 0.02 ml min^{-1})	0.42	1.46
wavelength (± 5 nm)	0.58	1.42
column oven temp (± 5.0°C)	0.74	1.35
pH of buffer (± 0.2)	1.54	0.28
organic composition (± 2)	0.29	1.46

Table 5: Robustness data for HCTZ and VAL.

analysis of HCTZ and VAL in pharmaceutical dosage form is precise, accurate, linear, robust and ultra fast. The shorter run time of 2 minute enables rapid determination of the drugs individually or in combination. The method was found to be specific and stability

	HCTZ	VAL
Time (hrs)	**Cumulative RSD%**	**Cumulative RSD%**
0	0.4	0.42
1	1.15	1.13
2	1.59	1.55
4	1.63	1.58
12	1.61	1.54
24	1.64	1.76

Table 6: UPLC chromatogram data for Solution Stability of HCTZ and VAL.

indicating as no interfering peaks of excipientswas noticed. Satisfactory results were obtained from validation of the method. This method exhibited an excellent performance in terms of sensitivityand speed. The method is more economical and suitable for laboratory use as solvent consumption is very less. Conventional reported HPLC methods may be replaced by the proposed UPLC method because of it's superiority in cost effectiveness, saving of analysis time per sample and better detection.

References

1. Swartz ME (2004) Ultra Performance Liquid Chromatography: Tommorrows HPLC technology today Lab Plus Int. 18: 6-9.
2. Michael ES, Brian JM (2004) Ultra performance liquid chromatography: tomorrow's HPLC technology today. Novel Phases for HPLC 1254-1256.
3. http://www.spectroscopyonline.com/spectroscopy/data/articlestandard/lcgc/242005/164646/article.pdf
4. Wren SAC, Tchelitcheff P (2006) Use of ultra-performance liquid chromatography in pharmaceutical development. Journal of Chromatography A 1119: 140-146.

5. Rao K, Jena N, RaoM (2010) Development and validation of a specific stability indicating high performance liquid chromatographic method for valsartan. J Young Pharm 2: 183-189.

6. Piao ZZ, Lee ES, Tran HT, Lee BJ (2008) Improved analytical validation and pharmacokinetics of valsartan using HPLC with UV detection. Arch Pharm Res 31: 1055-1059.

7. Ferreirosa N, Iriartea G, Alonso RM, Jiméneza RM (2007) Development of a solid phase extraction procedure for HPLC–DAD determination of several angiotensin II receptor antagonists in human urine using mixture design. Talanta 73: 748-756.

8. Satana, E, Altinay S, Goger NG, Ozkan SA, Senturk Z (2001) Simultaneous determination of valsartan and hydrochlorothiazide in tablets by first-derivative ultraviolet spectrophotometry and LC. J Pharm Biomed Anal 25: 1009-1013.

9. Gonzalez L, Lopez JA, Alonso RM, Jimenez RM (2002) Fast screening method for the determination of angiotensin II receptor antagonists in human plasma by high performance liquid chromatography with fluorimetric detection. J Chromatogr A 949: 49-60.

10. Shah NJ, Suhagia BN, Shah RR, Patel NM (2009) HPTLC method for the simultaneous estimation of valsartan and hydrochlorothiazide in tablet dosage form. Indian J Pharm Sci 71: 72-74.

11. Liu F, Zhang J, Gao S, Guo Q (2008) Simultaneous Determination of Hydrochlorothiazide and Valsartan in Human Plasma by Liquid Chromatography/ Tandem Mass Spectrometry. Analytical Letters 41: 1348-1365.

12. Lia H, Wang Y, Jiang Y,Tang Y, Wang J (2007) A liquid chromatography/tandem mass spectrometry method for the simultaneous quantification of valsartan and hydrochlorothiazide in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 852: 436-442.

13. Jothiewari D, Anandakumar K, Vijaya S (2010) Validated RP-HPLC method for simultaneous determination of amlodipine, valsartan and hydrochlorothiazide in bulk and in pharmaceutical formulation. Journal of pharmaceutical and biomedical sciences 5: 12.

14. Kharoaf M, Malkieh N, Murad M (2012) Tablet formulation and development of a validated stability indicating hplc method for quantification of valsartan and hydrochlorthiazide combination.International Journal of Pharmacy and Pharmaceutical Sciences 4: 284-290.

15. Hillaert S, Bossche W (2002) Optimization and validation of a capillary zone electrophoretic method for the analysis of several angiotensin-II-receptor antagonists; J Chromatogr A 979: 323-333.

16. HillaertS, De Beer TRM, De Beer JO, Van denBossche W (2003) Optimization and validation of a micellarelectrokinetic chromatographic method for the analysis of several angiotensin-II-receptor antagonists. J Chromatogr A 984: 135-146.

17. Tian D, Tian X, Tian T, Wang Z, Mo F (2008) Simultaneous Determination of Valsartan and Hydrochlorothiazide in Tablets by RP-HPLC. Indian J Pharm Sci 70: 372-374.

18. Simultaneous UV Spectrophotometric Determination of Valsartan and Amlodipine in tablet. International Journal of Chem Tech Research 2: 551-556.

19. Tian DF, Tian XL, Tian T, ZY Wang, Mo FK (2008) Simultaneous Determination of Valsartan and Hydrochlorothiazide in Tablets by RP-HPLC. Indian J Pharm Sci 70: 372-374.

20. ICH Q1A (R2), Stability Testing of New Drug Substances and Products, 2000.

21. ICH Q2 (R1), Validation of Analytical Procedures: Text and Methodology, 2005.