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Simultaneous determination of valsartan and hydrochlorothiazide in a tablet dosage form by liquid chromatography

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A selective, rapid and accurate liquid chromatographic method is described for the simultaneous determination of valsartan (VAT) and hydrochlorothiazide (HTZ) in a solid dosage form. The chromatographic separation of two drugs were achieved on Lichrosphere CN column 250 mm × 4.0 mm,5 µm, Merck-India. The mobile phase consisting mixture of methanol, isopropyl alcohol and n-hexane in the ratio of 50:25:25 v/v was delivered at a flow rate of 1.0 ml/min. Detection was performed at 265 nm. Linearity, accuracy and precision were found to be acceptable over the concentration range 40 to120 µg/ml for VAT and 6 to 18 µg/ml for HTZ, respectively. Seperation of VAT and HTZ were achieved in 5 min along with possible degradants. The proposed method can be used for the quantitative analysis of VAT and HTZ in formulation product.

Key words: Valsartan, hydrochlorothiazide, liquid chromatography.

INTRODUCTION

Valsartan (S)-N-[2'-(1H-tetrazole -5-yl) biphenyl-4-yl) methyl] valine is a potent, highly selective, orally active, specific angiotensin II receptor antagonist used as anti hypertensive drug. Hydrochlorothiazide, 6-chloro-3, 4-dihydro-2H -1, 2, 4-benzothiadiazine-7-sulphonamide-1, 1-dioxide, is a diuretic drug. The rationale behind this drug combination is that the treatment of hypertension in patients whose blood pressure is not adequately controlled by monotherapy, oral administration of valsartan with hydrochlorothiazide has been found more effective than use of either drug alone (Calhoun et al., 2008).

Several methods have been reported for the determination of valsartan and hydrochlorothiazide in combination. Medvedovici et al. (2000) reported a liquid extraction and high-performance liquid chromatography-diode array detector (HPLC-DAD) assay of hydrochlorotiazide from plasma for a bioequivalence study. Tian et al. (2008) developed a method for separation of valsartan (VAT) and hydrochlorothiazide (HTZ) using reversed-phase high-performance liquid chromatography (RP-HPLC) in isocratic mode. Ivanovic et al. (2007) employed RP-HPLC with gradient mode to monitor impurity level in

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valsartan and hydrochlorothiazide by RP-HPLC.

Satana et al. (2001) reported a method for simultaneous determination of valsartan and hydrochlorothiazide by first derivative UV spectrometry and LC method. Sevgi et al. (2002) reported comparison of UV-and second derivative spectrometric and RP-HPLC method for valsartan, using losartan as internal standard. Sioufil et al. (1994) automated determination of an angiotensin II receptor antagonist in plasma, Zendelovaska et al. (2004) developed solid extraction method for HTZ, Macek et al. (2006) developed rapid determination of VAT in human plasma by protein preparation and HPLC, Gonzalez et al. (2002) reported fast screening of angiotensin II receptor by flourimetric detection in human plasma, Iriarte et al. (2007) reported solid phase extraction-high performance liquid chromatography-ultra violet (SPE-HPLC-UV)-fluorimetric method for valsartan, Hillaert et al. (2003) reported simultaneous determination of HTZ and several angiotensin-II-receptor antagonist by capillary electrophoresis. Kadam et al. (2007) reported quantitative analysis of Valsartan and hydrochlorothiazide in tablets by high-performance thin layer chromatography with ultraviolet absorption densitometry; Koseki et al. (2007) developed LC-mass spectrometry method for quantification of VAT while Ramakrishna et al. (2005) developed LC-mass spectrometry method for quantification of HTZ in human plasma. Shah et al. (2009) reported LC-MS-MS method for simultaneous determination of VAT and HTZ. Vetuschi et al. (2005) fourth order UV derivative spectrophotometric methods was reported for estimation of either Irbesartan and HTZ or in combination. Sagrili et al. (2007) reported a method by cyano column using mixture of phosphate buffer, methanol and triethylamine at pH 2.5 for olmesartan and HTZ using VAT as internal standard. The European Pharmacopoeia, The United States Pharmacopeia (2007; 2008;2009) also reported HPLC method for VAT and HTZ in combination. The current method used was faster and equilibration time is less compared to the earlier cited methods. The column used was cyano column and mobile phase used was a mixture of N-hexane, methanol and Isoproropyl alcohol. Run time was just 5 minutes. All the degraded products of VAT and HTZ were well separated and method can be used as stability, indicating for both VAT and HTZ in bulk drug samples and in combined formulation in a shortest time.

METHODOLOGY

Chemicals and reagents

Valsartan and hydrochlorothiazide working standards were generous gifts from Torrent Pharmaceuticals Ltd (Ahmedabad, India)). Combination product of VAT and HTZ (Label claim valsartan 80 mg and Hydrochlorothiazide 12.5 mg), Valent-H (Lupin laboratories Ltd-India) and Valzaar–H (Torrent-India) were purchased from the market. Methanol, N-Hexane and isopropyl alcohol of HPLC grade were from Merck (India).

HPLC instrumentation and condition

The HPLC system consisted of Shimadzu LC-2010CHT with quaternary gradient pumps attached with UV and photodiode array (PDA) detector. The chromatographic separations were performed using lichrosorb cyano column 250 mm × 4.6 mm, 5 µcolumn maintained at 25°C using column oven eluted with mobile phase at the flow rate of 1.0 ml/min. The mobile phase consisted of a mixture of methanol, isopropyl alcohol and N-hexane (50:25:25) v/v filtered through 0.45 µm nylon filter and degassed in ultrasonic bath prior to use. Measurement made with injection volume was 5 µl and ultraviolet (UV) detection at 265 nm. The output signal was integrated using LC-solution soft ware.

Standard and sample preparation

The standard stock solutions 1000 µg/ml each of VAT and HTZ were prepared separately by dissolving working standards in methanol and diluted to desired volume with methanol. Standard calibration solution of VAT and HTZ having the concentration in the range of 40 to 120 µg/ml and 6.0 to 18 µg/ml, respectively were prepared by diluting stock solution with methanol.

Analysis of dosage form

Ten tablets were weighed, their mean weight determined and crushed in mortar. An amount of powdered mass equivalent to one tablet was transferred into a 100 ml volumetric flask containing 20 ml methanol, mechanically shaken for 10 min, ultrasonicated for 5 min and then diluted to volume with methanol and filtered. The first 10 ml of the filtrate was rejected, and the subsequence was used to prepare sample solution by diluting 10 ml of filtered solution to 100 ml with methanol.

RESULTS AND DISCUSSION

Method development

In an attempt to develop stability indicating assay method, under common conditions, these are applicable for the routine quality control of this product in ordinary laboratories and also can be used as stability. Mobile phase was selected in terms of binary mixtures of methanol and n-hexane (50:50 v/v) run on cyano column as a normal phase. After several trials it was found that the incorporation of isopropyl alcohol and decrease in n-hexane volume gives well defined peak of VAT and HTZ and retention time decreased from 10 to 3.5 min for HTZ and 7 to 1.5 min for VAT, which is eluting after dead volume. Finally mobile phase consisted of a mixture of methanol, n-hexane and isopropyl alcohol in the ratio of 50:25:25 v/v, which produced good resolution, reasonable retention and acceptable peak shape for both drugs. The tablet matrix was also determined to see if any interference from them existed. No significant peaks from matrix were observed in chromatogram, indicating no interference from the formulation matrix. A typical chromatogram for a tablet sample is shown in Figure 1. The retention time is 1.50 min for VAT and 3.50 min for HTZ, respectively. The run time is less than 5 min.

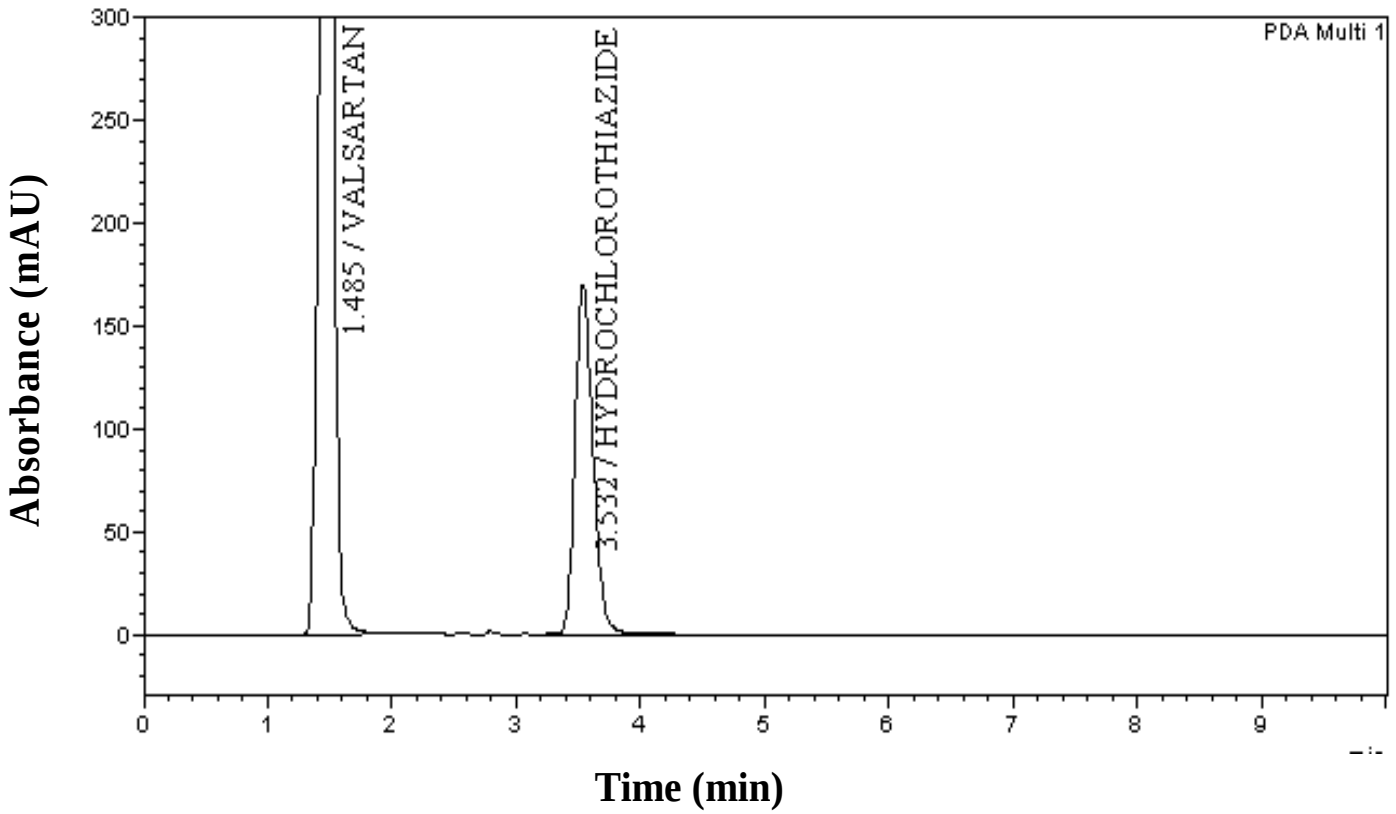

Figure 1. Typical chromatogram of VAT (1.485 min) and HTZ (3.532 min).

Degradation studies

Forced degradation of tablet samples under different stress condition (heat light, hydrogen peroxide, acid and base) were prepared for further evaluation of the selectivity of the proposed LC method. For preparing acid and base induced degradation product, 5 ml of 1 M HCl and 1 M NaoH were separately added to 80 mg valsartan and 12.5 mg hydrochlorothiazide equivalent tablet powder and exposed to 80°C for 6 h. The degraded samples were then neutralized and placed it in 100 ml volumetric flask and prepared as described in the sample preparation. For preparing, hydrogen peroxide induced degradation product 0.5 ml hydrogen peroxide (30% v/v) was added to 80 mg valsartan and 12.5 mg hydrochlorothiazide equivalent tablet powder, and exposed to 80°C for 6 h. The degraded sample was placed in 100 ml volumetric flask and prepared as described in the sample preparation. The forced degradation in acidic, basic and oxidation media was performed in the dark in order to avoid the possible effect of light. For preparing dry heat degradation product, 80 mg valsartan and 12.5 mg hydrochlorothiazide equivalent tablet powder was used and stored at 80°C for 6 h under dry heat condition in the dark and then cooled to room temperature. The degraded sample solution was prepared as described in the sample preparation. The photochemical stability of the drugs were also studied by exposing the tablet powder at 1,200 K lux of visible light and 200 W hm^{-2} of UV light by using photo stability chamber. The resulting solutions were used as the de-graded sample solution and determined under described chromatographic condition. A typical chromatogram of all degraded tablet sample are shown in Figures 2 to 6. The degraded samples were compared to a tablet sample without degradation. The spectral homogeneity (peak purity) 200 to 400 nm was determined in the forced degraded samples. The threshold was set at ≥ 0.990. The peak purity, peak threshold and percent degradation (Table 1) for VAT and HTZ in tablet were demonstrated that the proposed LC method was able to separate both drugs from degradants generated during forced degradation studies.

Linearity

The linearity of the response of two drugs were verified at five concentration level ranging from 40 to 120 µg/ml for VAT and 6.0 to 18 µg/ml for HTZ, respectively. The calibration curve was constructed by plotting mean area response A against concentration C of each drug. The regression equations obtained for the two drugs were A =

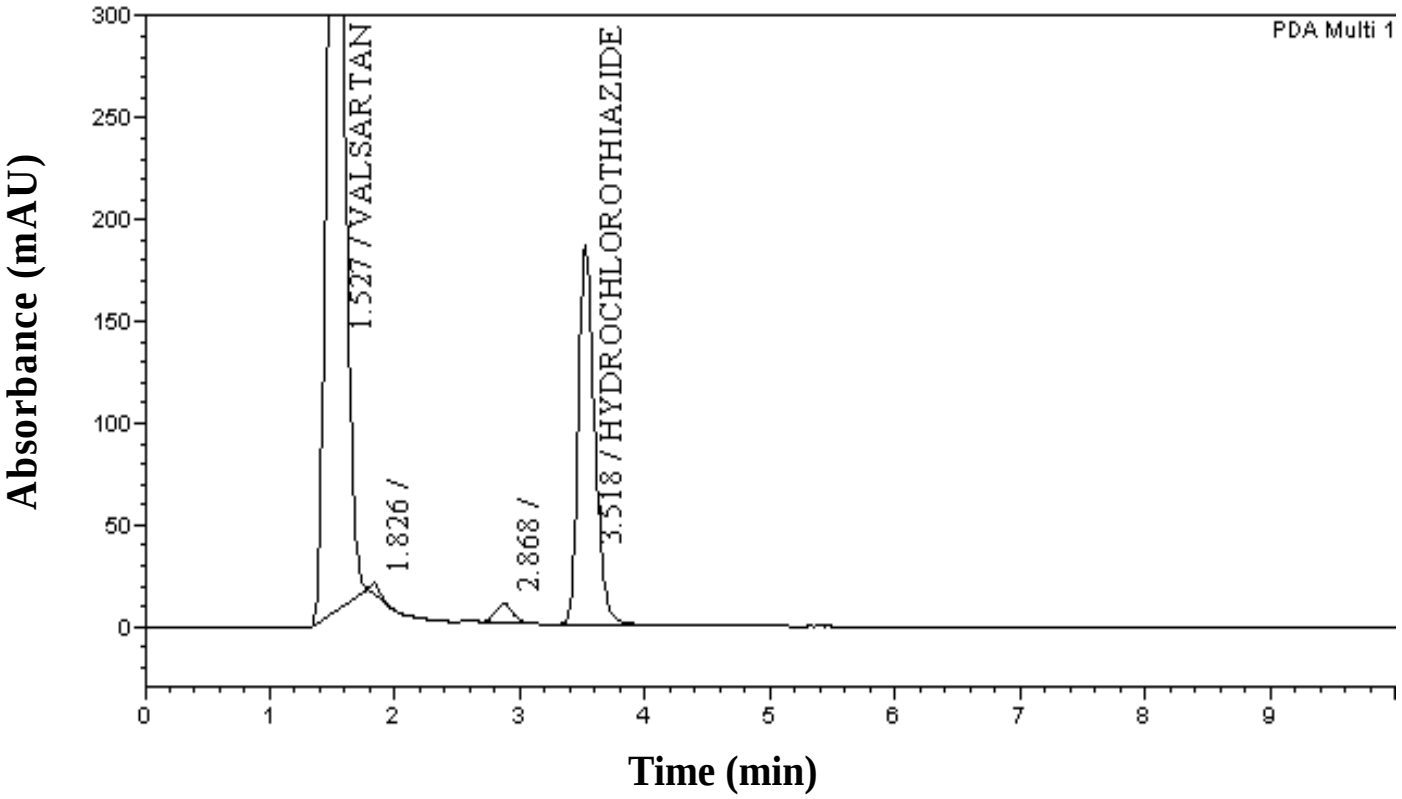

Figure 2. Chromatograms of acid hydrolysis–degraded VAT and HTZ n tablet sample.

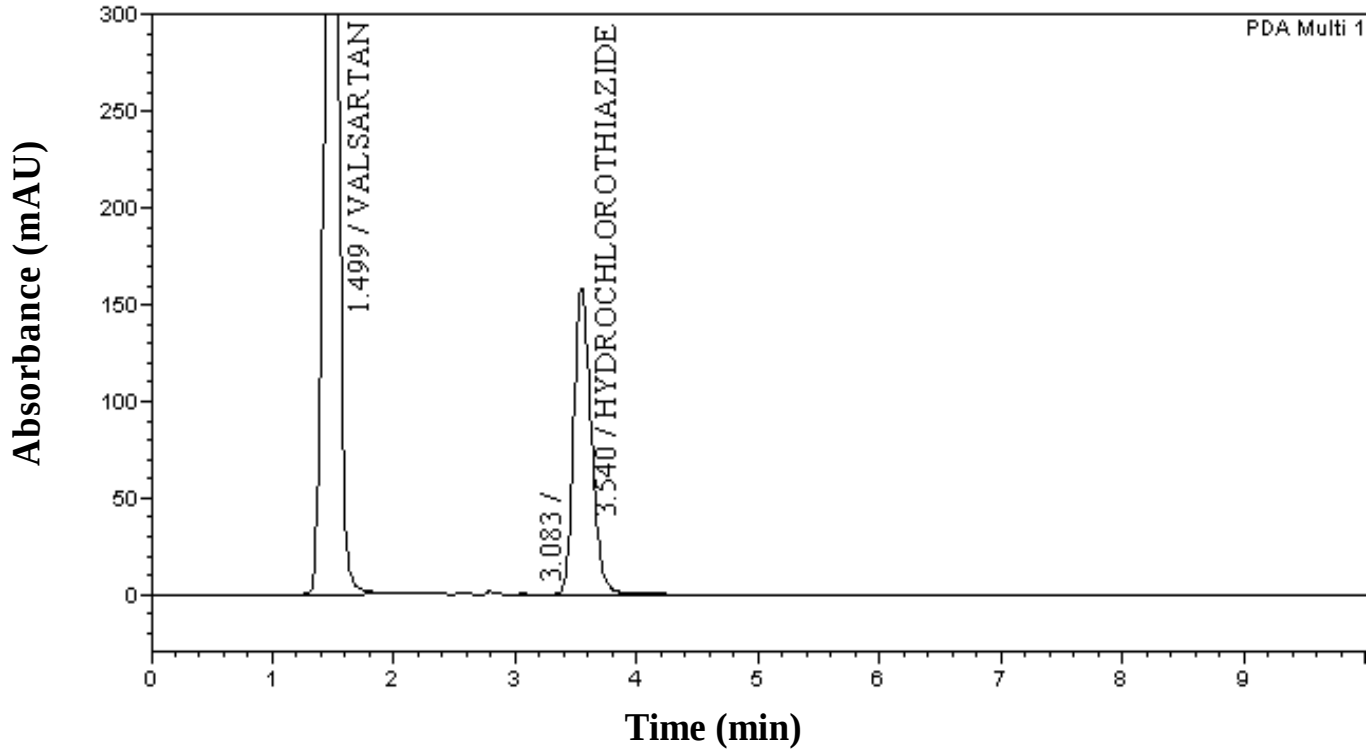

Figure 3. Chromatograms of base hydrolysis–degraded VAT and HTZ in tablet sample.

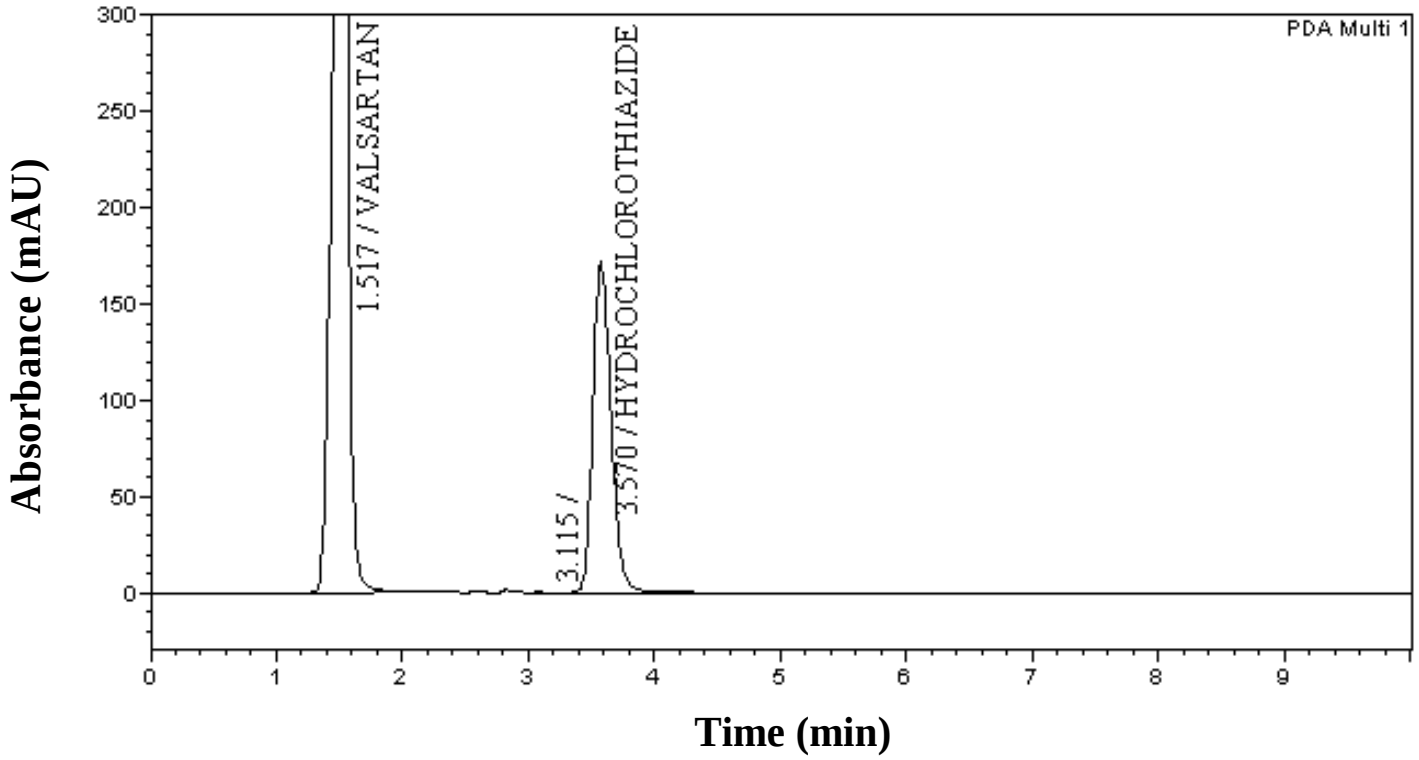

Figure 4. Chromatograms of oxidative–degraded VAT and HTZ in tablet sample.

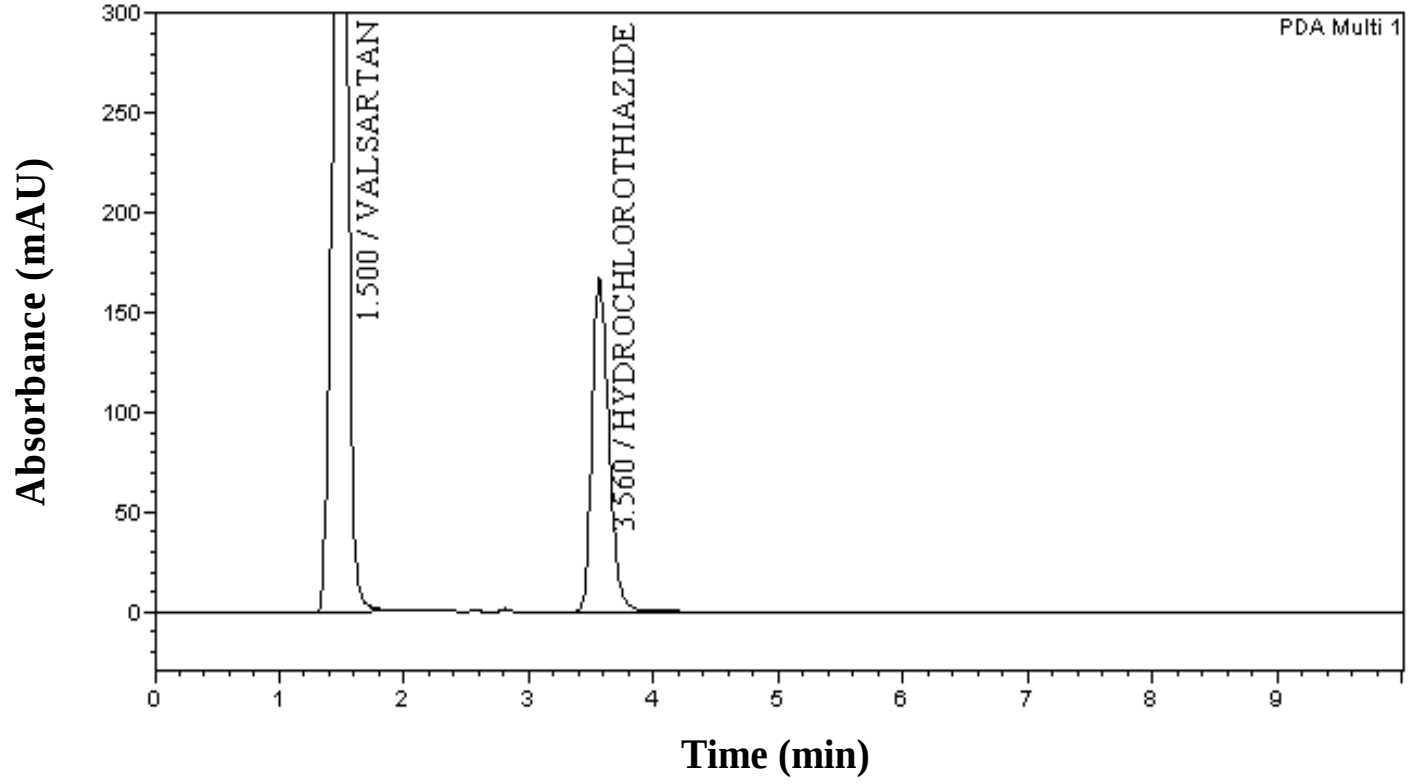

Figure 5. Chromatograms of thermal–degraded VAT and HTZ in tablet sample.

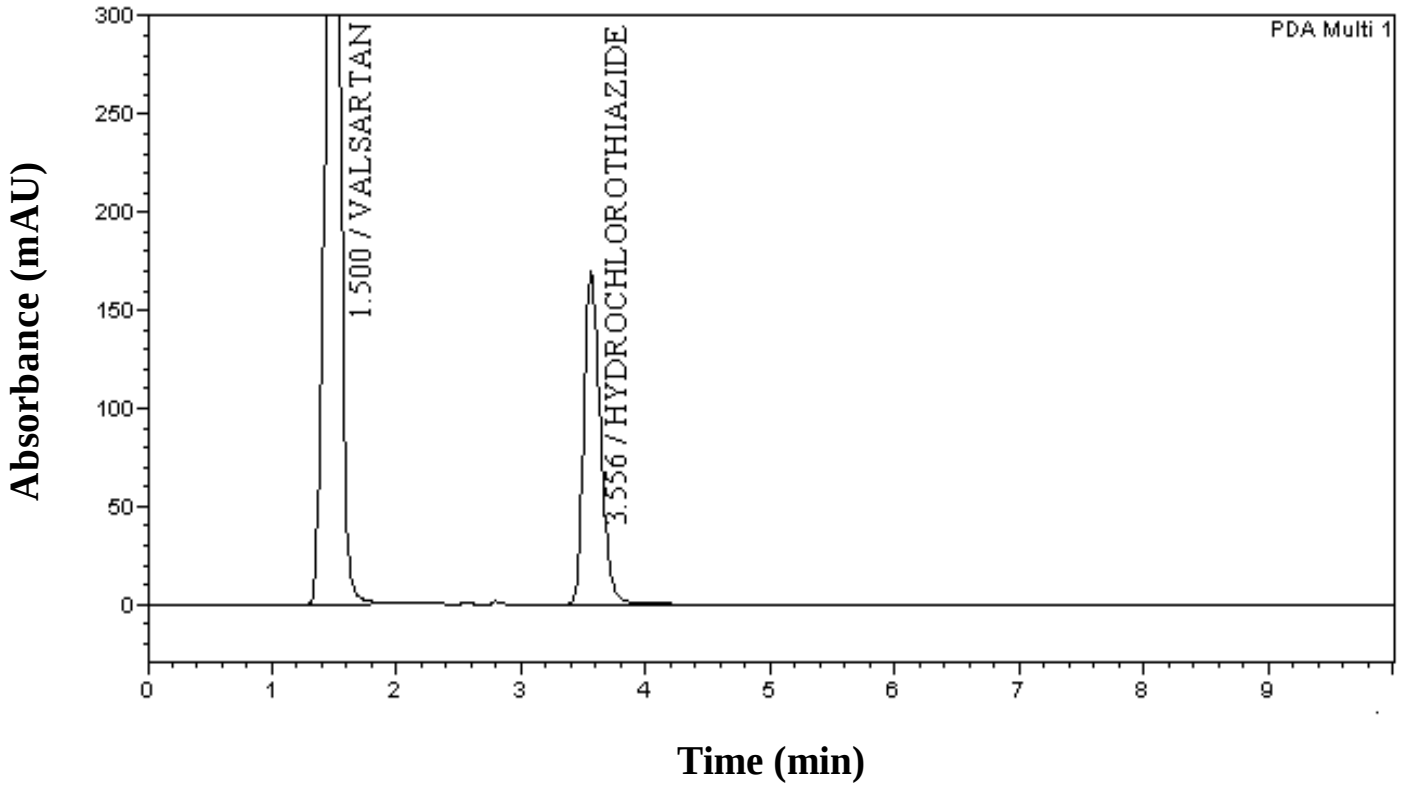

Figure 6. Chromatograms of photo degraded VAT and HTZ in tablet sample.

5000000C + 8708 (r = -0.9999, n = 5) for VAT and A = -2000000C + 214.4 (r = 0.9999, n = 5) for HTZ, respectively. The result shows that an excellent correlation existed between peak area and concentration of each drug within the concentration range tested.

Limit of quantitation

The limit of quantitation (LOQ) was defined as the lowest concentration that can be determined with acceptable accuracy and precision, which can be established at a signal to noise ratio of 10. LOQ of each drug was experimentally verified by six injections of each drug at its LOQ concentration. The LOQ of VAT and HTZ were found to be 0.15 and 0.20 µg/ml, respectively. The limit of detection (LOD) was defined as the lowest concentration that can be detected and established at a signal to noise ratio of 3. The LOD of VAT and HTZ were found to be 0.12 and 0.15 µg/ml. Table 2 represent linearity of the method.

Precision

Method repeatability (intra-day precision) was evaluated by assaying five samples prepared as described in the sample preparation. The mean % assay and percentage relative standard deviation (RSD) for assay values of VAT were found to be 98.5 and 1.16%, and for HTZ were 99.69 and 1.87%, respectively, which is well within the acceptance criteria that is, assay value should be between 97.0 and 103.0% and RSD should not be more than 2.0%. The intermediate precision (inter-day precision) was performed by assaying five samples prepared by different analyst, different HPLC systems and different HPLC column in different days as described in the sample preparation. The mean % assay and percentage RSD for assay values VAT were found to be 98.6 and 1.65% and for HTZ were 100.6 and 1.88%, respectively, which is well within the acceptance criteria. The results of intra-day precision and inter-day precision were evaluated with respect to student's t-test and found that t-test was passed. The result shows good precision of the method.

Accuracy

Accuracy was determined by applying the developed method to synthetic mixtures of excipients to which known amounts of each drug corresponding to 80, 100 and 120% of label claim had been added. The accuracy was then calculated as the percentage of analyte recovered

Table 1. Forced degradation results.

Stress condition	Peak purity		Single point threshold		% degraded
	VAT	HTZ	VAT	HTZ	
Light	1.0000	1.0000	0.9999	0.9999	0.0
Heat	1.0000	1.0000	0.9999	0.9999	0.0
Acid	1.0000	1.0000	0.9999	0.9999	0.1
Base	1.0000	1.0000	0.9999	0.9999	1.7
Oxidation	1.0000	1.0000	0.9999	0.9999	0.1

Table 2. Linearity, system suitability and accuracy parameters.

Parameter		VAT	HTZ
Linearity range		40 to120 µg/ml (n=5)	6 to18 µg/ml (n=5)
Correlation coefficient		0.9997	0.9997
Slope*		$2.22*10^{-7}$	$5.0*10^{-7}$
Intercept		-0.001958	-0.001511
Retention time*		1.5	3.5
Resolution factor*		-	7.50
Tailing factor*		0.91	1.14
%Accuracy* (Recovery studies)	80	101.5±1.7	102.8±1.5
	100	100.4±1.3	100.3±0.2
	120	101.6±1.3	102.0 ± 0.8

*Average of five readings ± standard deviation.

from the formulation matrix. Mean recoveries (Mean ± SD) for VAT and HTZ from the formulation are 102.2 ± 0.7% and 101.3 ± 0.4%, respectively. The inter-day accuracy was also determined by assaying the tablets in triplicate per day for consecutive 3 days. Mean recoveries for the inter-day accuracy are 101.2 ± 1.4% for VAT and 101.7 ± 0.8% for HTZ (Table 2). The obtained result suggested the accuracy of the developed method for the simultaneous determination of the two drugs in the formulation.

Robustness

The robustness of the method was determined by analyzing same sample at standard operating conditions and also by changing analytical conditions such as mobile phase composition, temperature and flow rate. In all, the deliberate varied chromatographic condition was carried out that is, organic phase composition, column temperature and flow rate in mobile phase. The system suitability parameter and % assay for the VAT and HTZ from the five replicate injections of test solution was found to be within the acceptable limits. The robustness of the method is established as the percentage deviation from the mean assay value obtained from precision study which is less than ± 2%. Table 3 represents the robustness of the method.

Assay of tablet

The validated LC method was applied to the determination of VAT and HTZ tablets. Two batches of the tablet were assayed and results are shown in Table 4, indicating that the amount of each drug in the tablet samples met with the requirements (90 to 110% of the tablet claim).

Conclusion

The developed LC method is sensitive, rapid, accurate, rugged and specific for the simultaneous determination of VAT and HTZ in a tablet formulation. None of the reported method so far is as quick as the current method, though it can be used for stability indicating study. Hence, the developed method is useful for the quality control of the formulation product as stability indicating method, with a run time of 5 min.

Table 3. VAT and HTZ assay robustness result.

Set name	Initial precision (RSD %)		Tailing factor (≤ 1.5)		Resolution (<2%)		%purity	
	VAT	HTZ	VAT	HTZ	VAT	HTZ	VAT	HTZ
Standard condition	1.5	1.8	0.91	1.14	-	7.89	98.3	100.64
Mobile phase organic composition (45:30:25)	0.9	0.6	0.87	1.03	-	7.50	97.78	98.45
Mobile phase organic composition (55:22.5:22.5)	0.9	1.0	0.89	1.04	-	7.60	97.34	98.25
Mobile phase organic composition (45:22.5:32.5)	0.9	0.8	0.89	1.04	-	7.01	97.34	98.25
Column temperature (+5°C)	0.5	0.6	0.88	1.14	-	7.49	97.77	99.39
Column temperature (-5°C)	0.1	0.1	0.91	1.14	-	7.83	97.9	99.00
Flow rate (+10%)	0.5	0.6	0.91	1.13	-	7.66	97.71	99.18
Flow rate (-10%)	0.3	0.6	0.92	1.14	-	7.99	97.42	99.01

Table 4. Result of VAT and HTZ in marketed formulation

Marketed formulation	Drug	% Amount found ± SD	% RSD
Valent-H	Valsartan	99.37 ± 0.62	0.62
	Hydrochlorothiazide	100.23 ± 0.10	0.10
Valzaar -H	Valsartan	101.27 ± 0.10	0.10
	Hydrochlorothiazide	100.42 ± 0.43	0.43

RSD: relative standard deviation.

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