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Research Article

New Stability Indicating RP-HPLC Method for the Quantification of Hydrochlorothiazide and Valsartan Tablets

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Abstract

A new simple isocratic reverse phase liquid chromatographic method has been developed for the quantification of Hydrochlorothiazide and Valsartan in pharmaceutical formulations (Tablets). Shimadzu Model CBM-20A/20 Alite HPLC system equipped with PDA detector with Enable C18 (150 mm × 4.6 mm i.d, 5 µm particle size) was used with a mobile phase mixture of 0.1% Acetic acid and Methanol (20:80 v/v) and flow rate of 0.8 mL/min (Detection wavelength 260 nm) for the simultaneous estimation of Hydrochlorothiazide and Valsartan. Hydrochlorothiazide and Valsartan obey Beer-Lambert's law over the concentration range 1-30µg/ml with regression equations y = 70500x - 8392.8 (R^2 = 0.9992) and y = 39602x – 6449 (R^2 = 0.9995) respectively. The LOD and LOQ were found to be 0.3078 µg/ml and 0.9345 µg/ml for Hydrochlorothiazide and 0.3189 µg/ml and 0.9672 Valsartan respectively. Forced degradation studies were performed for the combined dosage forms and the method was validated. The method is found to be precise, accurate and robust and is suitable for the routine quality control applications in pharmaceutical formulation.

Keywords: Hydrochlorothiazide; Valsartan; RP-HPLC; Forced Degradation Studies; Validation

Introduction

Hydrochlorothiazide (HCT) is a diuretic (Figure 1) and it is chemically, 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide ($C_7H_8ClN_3O_4S_2$) [1]. Valsartan (VAL) is an angiotensin II receptor antagonist used in treatment of high blood pressure (Figure 2) and it is chemically, N-(1-oxopentyl)-N- [[2 '-(1H-tetrazol-5-yl) [1,1 '-biphenyl]-4-yl] methyl]-L-Valine ($C_{24}H_{29}N_5O_3$; 435.5 gm/mole) [2,3]. The combination of Hydrochlorothiazide and Valsartan is used for the treatment of hypertension. The simultaneous determination of Hydrochlorothiazide and Valsartan was performed by various analytical techniques such as liquid chromatography [4-9], HPTLC (10), TLC (11) and spectrophotometry [12-21] in pharmaceutical dosage forms and in the present study a simple and robust isocratic reverse phase liquid chromatographic method has been developed for the simultaneous determination of Valsartan and Hydrochlorothiazide tablets and the method was validated as per ICH guidelines [22]. Forced degradation studies were performed for the combined dosage forms and the method was validated [23].

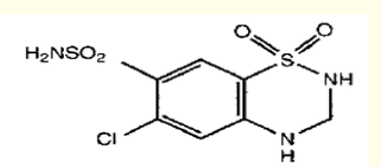

Figure 1: Chemical structure of Hydrochlorothiazide.

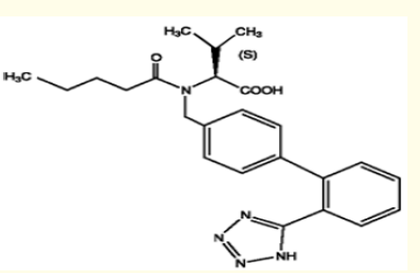

Figure 2: Chemical structure of Valsartan.

Materials and Methods

Shimadzu Model CBM-20A/20 Alite HPLC system equipped with PDA detector with Enable C18 (150 mm × 4.6 mm i.d, 5 µm particle size) was used with a mobile phase mixture of 0.1% Acetic acid and Methanol (20:80 v/v) and flow rate of 0.8 mL/min (Detection wavelength 260 nm) The combination of Hydrochlorothiazide and Valsartan is available as tables with brand names VALZAAR-H (Torrent Pharmaceuticals Ltd) and CO-DIOVAN (Novartis India Ltd), VALENT–H (LUPIN Ltd), VALEMBIC –H (Alembic Pharmaceuticals Ltd) (Labelled claim: Valsartan 80 mg and Hydrochlorothiazide 12.5 mg) etc.

Preparation of stock and working standard solution

Accurately 10 mg of each of Hydrochlorothiazide and Valsartan were weighed and transferred to clean and dry 10 ml volumetric flasks separately and dissolved in HPLC grade methanol (1000 µg/mL) and working standard solutions were prepared on dilution with mobile phase consisting of 0.1% Acetic acid and Methanol (20:80 v/v) (100 µg/mL) from the stock solutions.

Method validation

Linearity

A series of Hydrochlorothiazide and Valsartan solutions were prepared with mobile phase mixture consisting of 0.1% Acetic acid and Methanol (20:80 v/v) from the stock solution and 20 µl was injected in to the HPLC system. The peak area and there by the mean peak area values of these solutions were calculated from the respective chromatograms of these two drugs and calibration curves were drawn by taking the concentration of the drug on the x axis and the corresponding mean peak area values on the y axis.

Precision and accuracy studies

The ability of the method to give reliable data was checked in terms of repeatability (Intraday) and intermediate precision (Inter day). Sample solution prepared from tablets was suitably diluted to give concentrations at three different levels within the linearity range. The solutions at each concentration were prepared in triplicate and each solution was injected thrice. The assay obtained from the peak areas was calculated and the % RSD was analyzed. Accuracy of the method was determined by standard addition method by adding working standard solutions of Hydrochlorothiazide and Valsartan (prepared in the ratio of the formulation) to a pre analyzed sample solution at three levels i.e. 80, 100 and 120%. The percentage recovery was calculated at each level and the % RSD was calculated.

Robustness

Robustness is a measure of the capability of a method to remain unaffected by small, but deliberate variations in method parameters. The robustness parameters validated under the heads flow rate, pH and mobile phase composition. The standard and sample solutions prepared within the linearity range were injected with flow rates 0.8 ± 0.1 mL/min; pH 3.0 ± 0.1; mobile phase ratio by ± 2.0 (18:82 and 22:78 of acetic acid: methanol) from the standardized assay.

Force degradation studies

Acid degradation

The combined solution of Hydrochlorothiazide and Valsartan was transferred to a 10ml volumetric flask, 1ml of 0.1N HCL was added and refluxed at 70°C for 1hr. The solution was cooled to room temperature and neutralized with 0.1N NaOH. The solution was further diluted up to the mark with diluents. A 20 µL of this solution was injected thrice and analyzed.

Alkali degradation

The combined solution of Hydrochlorothiazide and Valsartan was transferred to a 10ml volumetric flask, 1ml of 0.1N NaOH was added and refluxed at 70°C for 1hr. The solution was cooled to room temperature and neutralized with 0.1N HCL. The solution was further diluted up to the mark with diluents. A 20 µL of this solution was injected thrice and analyzed.

Oxidative degradation

The combined solution of Hydrochlorothiazide and Valsartan was transferred into a 10ml volumetric flask, 1ml of Hydrogen Peroxide was added and refluxed at 70°C for 1hr. The solution was cooled to room temperature and diluted up to the mark with diluents. A 20 µL of this solution was injected thrice and analyzed.

Thermal degradation

The combined solution of Hydrochlorothiazide and Valsartan was transferred into a 10ml volumetric flask, the solution was refluxed at 70°C for 1hr.The solution was cooled at room temperature and diluted up to the mark with diluents. A 20 µL of this solution was injected thrice and analyzed.

Photolytic degradation study

The combined solution of Hydrochlorothiazide and Valsartan was transferred into a 10ml volumetric flask and the solution was exposed to the UV light at shorter wavelength of 254 nm for 1hr. The solution was diluted up to the mark with diluents. A 20 µL of this solution was injected thrice and analyzed.

Application of the method to the marketed formulations (Tablets)

Twenty tablets of two different brands containing Valsartan 80 mg and Hydrochlorothiazide 12.5 mg were weighed accurately, powdered and extracted with methanol separately in two different volumetric flasks. Dilutions were made using mobile phase and 20 µL of these solutions were injected thrice and the percentage recovery of each drug was calculated.

Result and Discussion

Method validation

A new isocratic RP-HPLC method has been developed for the simultaneous determination of Hydrochlorothiazide and Valsartan in pharmaceutical formulations. Mobile phase mixture consisting of 0.1% Acetic acid and Methanol (20:80 v/v) with flow rate of 0.8 mL/min (Detection wavelength 260 nm) are the optimized chromatographic conditions chosen for the simultaneous estimation of Hydrochlorothiazide and Valsartan. Hydrochlorothiazide and Valsartan obey Beer-Lambert's law over the concentration range 1-30µg/ml with regression equations y = 70500x - 8392.8 (R^2 = 0.9992) and y = 39602x – 6449 (R^2 = 0.9995) respectively. The LOD and LOQ were found to be 0.3078 µg/ml and 0.9345 µg/ml for Hydrochlorothiazide and 0.3189 µg/ml and 0.9672 Valsartan respectively. Both drugs obey Beer-Lambert's law over the concentration

range 1-30 µg/ml (Table 1) and the calibration curves were shown in Figure 3 and Figure 4 for Hydrochlorothiazide and Valsartan respectively.

Conc. (µg/mL)	Hydrochlorothiazide		Valsartan	
	Peak area	Mean ± SD (%RSD)	Peak area	Mean ± SD (%RSD)
1	69592	69466.5 ± 177.2 (0.25)	39308	3891.5 ± 552.2 (1.4)
	69341		38527	
5	339760	339356.5 ± 570.6 (0.16)	187389	185821 ± 2217 (1.19)
	338953		184253	
10	690108	691121.5 ± 1433.3 (0.2)	384045	385793 ± 2472 (0.64)
	692135		387541	
15	1038624	1038259 ± 516 (0.04)	576749	571586 ± 7301 (1.2)
	1037894		566423	
20	1416183	794998 ± 2182 (0.07)	793455	794998 ± 2182 (0.27)
	1415894		796541	
25	1710370	1711758 ± 1962 (0.11)	968887	965217 ± 518 (0.53)
	1713145		961548	
30	2141221	2142940 ± 2430 (0.11)	1196356	1197404 ± 1481 (0.12)
	2144658		1198451	

Table 1: Linearity of Hydrochlorothiazide and Valsartan.

*Mean of three determinations.

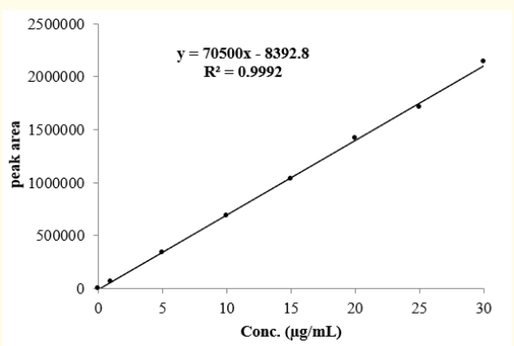

Figure 3: Calibration curve of Hydrochlorothiazide.

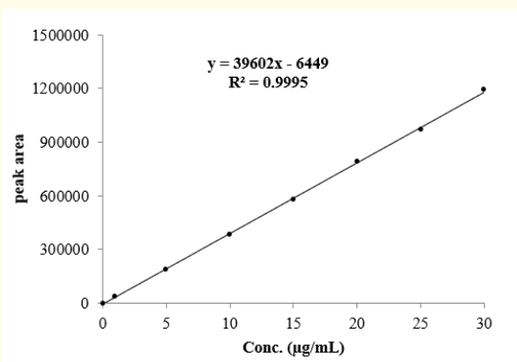

Figure 4: Calibration curve of Valsartan.

The percentage RSD in precision (Table 2 and Table 3), accuracy (Table 4) and robustness (Table 5) study was found to be less than 2% indicating that the proposed method is precise, accurate and robust. System suitability parameters has shown that the theoretical plates are > 2000 (Hydrochlorothiazide: 1.1 and Valsartan: 1.4), the tailing factor was found to be less than 1.5 (Hydrochlorothiazide: 5796 and Valsartan: 7432) and the resolution was greater than 2.

Conc. (µg/mL)		Mean peak area		Assay (% w/w)		*Mean ± SD (% RSD)	
HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL
2.5	16	167456	598861	99.77	99.53	98.68 ± 1.6 (1.08)	98.16 ± 0.33 (0.4)
2.5	16	165407	595774	98.61	98.04		
2.5	16	163720	594920	97.65	98.91		
3.75	24	222827	806693	99.46	98.55	99.64 ± 0.28 (0.33)	98.63 ± 0.21 (0.25)
3.75	24	224178	805888	99.97	98.47		
3.75	24	222926	809754	99.5	98.88		
5	32	292416	1078770	99.34	98.63	99.54 ± 0.40 (0.47)	98.41 ± 0.22 (0.26)
5	32	292213	1073183	99.28	98.19		
5	32	294761	1075908	98	98.41		

Table 2: Intraday precision study.

Conc. (µg/mL)		Peak area		Assay (% w/w)		*Mean ± SD (% RSD)	
HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL
2.5	16	165326	596872	98.77	99.24	98.68 ± 1.9 (1.58)	98.16 ± 1.2 (1.4)
2.5	16	165407	595982	98.61	98.43		
2.5	16	165610	596920	97.65	98.10		
3.75	24	225827	806893	97.46	98.91	98.64 ± 1.58 (1.5)	98.63 ± 1.21 (1.25)
3.75	24	224548	805388	98.97	98.12		
3.75	24	222736	809354	98.5	98.47		
5	32	282526	1074189	98.34	96.61	99.54 ± 1.40 (1.47)	97.8 ± 1.22 (1.26)
5	32	282247	1073161	98.28	97.24		
5	32	284761	1071504	98	97.19		

Table 3: Inter day precision study.

*Mean of three determinations.

Assay of Hydrochlorothiazide and Valsartan tablets

The percentage of purity of Hydrochlorothiazide and Valsartan tablets was found to be 99.24-99.45 for Valsartan and 99.28-99.44 for Hydrochlorothiazide respectively (Table 6). The respective chromatograms observed for the placebo and for one of the marketed formulations was shown in Figure 5 and Figure 6 respectively.

Forced degradation studies

Both Hydrochlorothiazide and Valsartan were found to be very resistant towards acidic, alkaline, oxidative, thermal and photolytic degradations. The percentage decomposition was found to be less

Level	Tablet (µg/mL)	Pure drug (µg/mL)			Mean peak area		Conc. found (µg/mL)		Recovery (%)		*Mean ± SD (% RSD)	
	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL
80%	2	12.8	1.6	10.24	260536	935069	3.58	23.45	99.35	101.77	100.51 ± 1.09 (1.02)	101.37 ± 0.37 (0.37)
	2	12.8	1.6	10.24	264589	928541	3.63	23.28	100.94	101.06		
	2	12.8	1.6	10.24	265347	930147	3.64	23.32	101.24	101.23		
100%	2	12.8	2	12.8	289485	1028382	3.99	25.81	99.68	100.80	100.02 ± 0.3 (0.37)	100.06 ± 0.98 (0.98)
	2	12.8	2	12.8	291542	1024587	4.02	25.71	100.41	100.43		
	2	12.8	2	12.8	290358	1009547	4.00	25.33	99.99	98.94		
120%	2	12.8	2.4	15.36	320696	1108221	4.43	27.82	100.68	98.80	100.68 ± 0.25 (0.24)	98.91 ± 0.43 (0.43)
	2	12.8	2.4	15.36	321489	1114796	4.44	27.99	100.93	99.39		
	2	12.8	2.4	15.36	319964	1105478	4.42	27.75	100.44	98.55		

Table 4: Accuracy study.

*Mean of three determinations.

Parameter	Condition	Sample peak area		Tailing factor		Tailing factor		Theoretical plates		% Assay	
		HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT	VAL
Flow rate (± 0.1 mL/min)	0.7	1784624	976481	1781812	970913	1.3	1.8	8568	7565	101.1	100.2
				1725643	973567						
	0.8	1668146	961828	1643824	967029	1.3	1.8	9460	8180	99.8	99.9
				1623045	965888						
	0.9	1525694	958421	1520244	957622	1.4	1.9	8494	7272	98.5	98.1
				1590630	957471						
Mobile phase ratio (± 2%)	22:78	1741984	976475	1744757	970813	1.04	1.2	8788	8361	100	100.1
				1743024	971567						
	20:80	1668146	961828	1643824	967029	1.3	1.8	9460	8180	99.8	99.9
				1623045	965888						
	18:82	1554879	965411	1539130	952422	1.4	1.6	9137	8082	98.9	98.3
				1543824	951471						
Mobile phase pH (± 0.10)	3.4	1458769	922736	1410630	925426	1.5	1.6	7283	8145	98.1	98.5
				1474457	924213						
	3.5	1668146	961828	1643824	967029	1.3	1.8	9460	8180	99.8	99.9
				1623045	965888						
	3.6	1668417	968427	1648439	959324	1.2	1.4	7649	7945	98.2	99.01
				1645968	964845						

Table 5: Robustness study.

*Mean of three determinations.

Brand name	Drug	Label claim (mg)	Amount obtained (mg)	*Assay (%w/w)
Brand I	VAL	80	79.39	99.24
	HCTZ	12.5	12.43	99.44
Brand II	VAL	80	79.56	99.45
	HCTZ	12.5	12.41	99.28

Table 6: Assay of Valsartan and Hydrochlorothiazide tablets.

* Mean of three determinations.

Stress conditions	*(%) Drug recovered		* (%) Drug decomposed	
	HCT	VAL	HCT	VAL
Standard Drug	100	100	-	-
Acidic degradation	99.12	98.36	0.78	1.64
Alkaline degradation	99.64	99.58	0.36	0.42
Oxidative degradation	100.01	97.91	-0.01	2.09
Thermal degradation	99.26	99.46	0.74	0.54
Photolytic degradation	99.01	99.82	0.99	0.18

Table 7: Forced degradation studies of Valsartan and Hydrochlorothiazide.

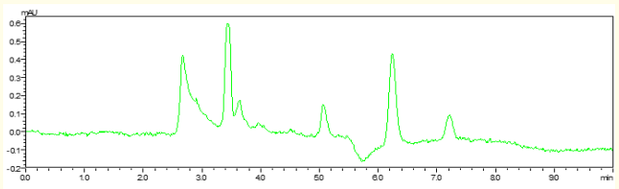

Figure 5: Chromatogram of the placebo.

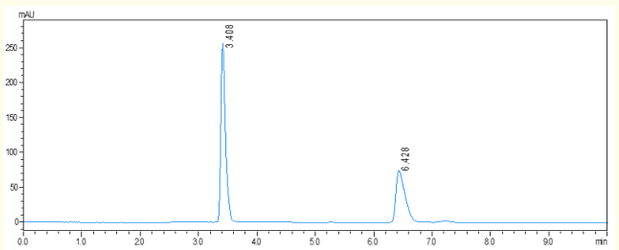

Figure 6: Chromatogram of Hydrochlorothiazide (Rt 3.408 min) and Valsartan (Rt 6.428 min) obtained during assay.

Figure 7: Overlay chromatogram of Hydrochlorothiazide (Rt 3.4 min) and Valsartan (Rt 6.4 min) A) Acidic degradation B) Basic degradation C) Oxidative degradation D) Thermal degradation E) Photolytic degradation F) Formulation G) API.

than 2% and almost it is negligible for the given stress degradation conditions applied. The results were given in Table 7 and the respective chromatograms obtained were shown in Figure 7. Specificity of the method was established based on the studies which indicated that there is no interference from the excipients, impurities, degradation products.

Conclusion

The proposed stability indicating liquid chromatographic method was found to be simple, precise, accurate and robust for the routine analysis of Hydrochlorothiazide and Valsartan tablets. The method is found to be selective and specific and during the degradation studies the percentage of drug decomposed was less than 2% and no degradants were found during the study.

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