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Original Article

Stability indicating HPLC method for simultaneous estimation of hydrochlorothioazide, amlodipine and valsartan in pharmaceutical dosage form

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ABSTRACT

A new HPLC method was developed and validated for the determination of HCTZ, Amlodipine, Valsartan in tablet dosage form. The chromatographic separation was achieved on a Inertsil ODS 3V(4.0 X 100mm, 5µ) with a mobile phase combination of 0.1% TFA buffer and Acetonitrile (gradient) at a flow rate of 1.0 ml/min, and the detection was carried out by using UV detector at 251nm. The total run time was 10 minutes. The retention time of HCTZ, Amlodipine, Valsartan were found to be 1.58 min., 3.28min., and 5.65 min. respectively. The performance of the method was validated according to the present ICH guidelines.

Key words: HPLC, Hydrochlorothioazide, Amlodipine and valsartan

INTRODUCTION

Hydrochlorothiazide is antihypertensive agent. It is used for management of hypertension and angina pectoris[1]. Chemical name of Hydrochlorothiazide is 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzo thiadiazine-7-sulfonamide. Hydrochlorothiazide is a prototype drug of thiazide diuretics. It reduces the reabsorption of electrolytes from the renal tubules. This results in increased excretion of water and electrolytes, including sodium, potassium, chloride, and magnesium. It has been used in the treatment of several disorders including edema, hypertension, diabetes insipidus, and hypoparathyroidism. Hydrochlorothiazide, a thiazide diuretic, inhibits water reabsorption in the nephron by inhibiting the sodium-chloride symporter (SLC12A3) in the distal convoluted tubule, which is responsible for 5% of total sodium reabsorption[2].

Amlodipine is a long-acting 1,4-dihydropyridine calcium channel blocker.Chemical name of Amlodipine is 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate.It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. By inhibiting the influx of calcium in smooth muscle cells, amlodipine prevents calcium-dependent myocyte contraction and vasoconstriction. A second proposed mechanism for the drug's vasodilatory effects involves pH-dependent inhibition of calcium influx via inhibition of smooth muscle carbonic anhydrase. Some studies have shown that amlodipine also exerts inhibitory effects on voltage-gated N-type calcium channels. N-type calcium channels located in the central nervous system may be involved in nociceptive signaling and pain sensation. Amlodipine is used to treat hypertension and chronic stable angina[3].

Valsartan is an angiotensin-receptor blocker (ARB). Chemical name of Valsartan is (2S)-3-methyl-2-[N-({4-[2-(2H-1,2,3,4-tetrazol-5-yl) phenyl]phenyl}methyl)pentanamido]butanoic acid. Valsartan is used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy and heart failure. Valsartan lowers blood pressure by antagonizing the renin-angiotensin-aldosterone system (RAAS); it competes with angiotensin II for binding to the type-1 angiotensin II receptor (AT1) subtype and prevents the blood pressure increasing effects of angiotensin II. Unlike angiotensin-converting enzyme (ACE) inhibitors, ARBs do not have the adverse effect of dry cough. Valsartan may be used to treat hypertension, isolated systolic hypertension, left ventricular hypertrophy and diabetic nephropathy. It may also be used as an alternative agent for the treatment of heart failure,

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systolic dysfunction, myocardial infarction and coronary artery disease[4].

The literature survey revealed that there are few HPLC and spectroscopic methods available for the determination of HCTZ, Amlodipine, Valsartan in pure and combined dosage forms. The present study was aimed to develop a new HPLC method for simultaneous estimation of HCTZ, Amlodipine, Valsartan in combined pharmaceutical dosage form.

EXPERIMENTAL

Chemicals and reagents: HCTZ, Amlodipine, Valsartan bulk drugs were made available from Pharmatrain, Kukatpally, Hyderabad.Methanol, Acetonitrile were obtained from Standard reagents. Commercially available Exforge HCT was used for the dosage form analysis. All chemicals and reagent used were of HPLC grade, Milli-Q-water was used throughout the experiment.

Equipments: The Waters HPLC system with a UV or photo diode array detector was used for method development and validation. The output signal was monitored and processed by using Empower software.

Chromatographic condition: The mobile phase used was mixture of 0.1% TFA buffer and Acetonitrile in gradient elution at a flow rate of 1.0 ml/min. The analytical column used Inertsil ODS 3V (4.0 X 100mm, 5µ) The detection was carried out at a wavelength of 251nm for a run time of 10 min. Diluent used buffer and Acetonitrile in the ratio of 70:30 v/v

Gradient programme

Time	Mobile phase-A (%)	Mobile phase-B (%)
0.0	70	30
3.0	50	50
7.0	30	70
7.5	70	30
10.0	70	30

Preparation of standard solution: Accurately weigh and transfer about 50 mg, 50mg, 160mg of HCTZ and Amlodipine and Valsartan working standard respectively into a 100 ml, 100ml, 25ml volumetric flask individually add methanol and sonicate to dissolve it completely and make volume up to the mark with the methanol. Further pipette 5.0 ml of HCTZ, 2.0 ml of Amlodipine & 5.0ml of Valsartan from the above stock solution into a 100ml volumetric flask and dilute up to the mark with diluent.

Assay of Pharmaceutical Dosage form: (Sample Preparation): Twenty tablets of Exforge HCT were weighed to get the average weight and then ground. An amount of powder equivalent to 25 mg, 10mg, 320mg of HCTZ and Amlodipine and Valsartan Tablet powder respectively into a 250 ml volumetric flask individually add methanol and sonicate to dissolve it completely and make volume up to the mark with the same solvent.Further pipette 5.0 ml of above stock solution into a 20ml volumetric flask and dilute up to the mark with diluent.

RESULTS AND DISCUSSION

Method development: Chromatographic parameters were preliminary optimized to develop HPLC method for simultaneous estimation of HCTZ, Amlodipine, Valsartan with short analyses time (10min), and acceptable resolution (> 2). The isoabsortive point of HCTZ, Amlodipine, Valsartan selected was 251 nm. In order to identify a suitable organic modifier, various compositions of acetonitrile and methanol were tested along with 0.1% TFA buffer. Different columns like X-terra, Inertsil, Inspire columns were tried. Resolution was the major problem while we are developing method. Resolution was less very less when we are using one organic phase, to increase resolution 0.1%TFA and acetonitrile were used in gradient mode.

Finally separation for simultaneous determination of HCTZ, Amlodipine, Valsartan was carried out by gradient elution with a flow rate of 1.0 mL/min in Inertsil ODS 3V(4.0 X 100mm, 5µ) The standard chromatogram was shown in Fig-1.The system suitability parameters were shown in Table-1.

Fig.1: Standard chromatogram

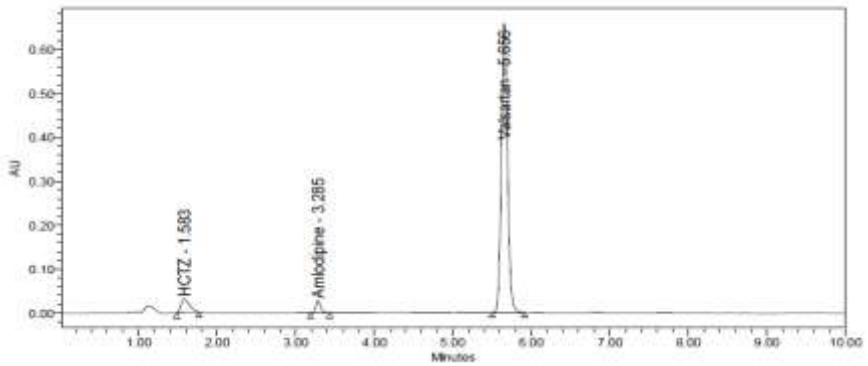


Table 1:system suitability parameters

Parameter	HCTZ	Amlodipine	Valsartan
Retention time	1.58	3.28	5.65
USP Tailing	1.42	1.12	1.10
USP Resolution		10.42	17.99

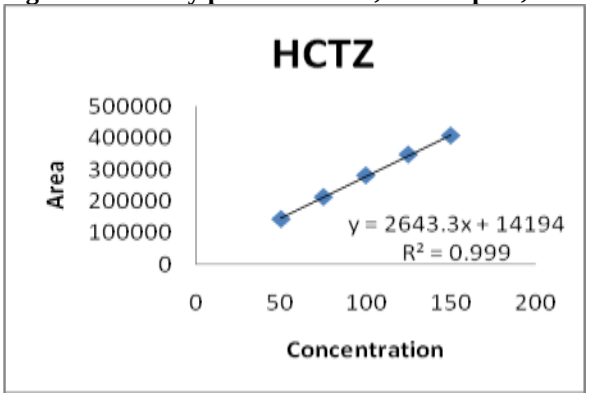
Method Validation: The above method was validated according to ICH guidelines to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method[5].

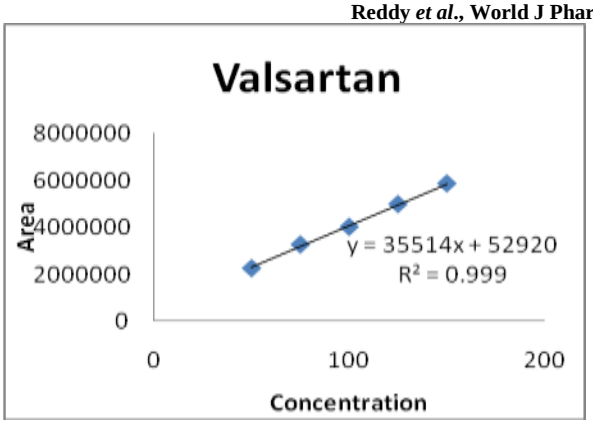
Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity of detector response for HCTZ, Amlodipine, Valsartan was established by analyzing serial dilutions of a stock solution of the working standard. Five concentrations ranging from 12.5-37.5 µg/ml for HCTZ, 5-15 µg/ml for Amlodipine, 160-480 µg/ml for Valsartan were prepared and analyzed. The linearity graph was plotted using concentration Vs peak area and they were shown in Fig-1.2. Slope, correlation coefficient (R) and intercept were calculated and the results were shown in Table-2.

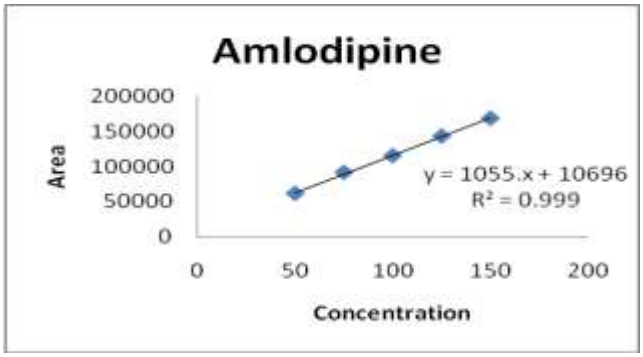
Table 2: Regression and statistical parameters

Parameter	HCTZ	Amlodipine	Valsartan
ConcentrationRange (µg/ml)	12.5-37.5	5-15	160-480
Correlation coefficient	0.999	0.999	0.999
Intercept	14194	10696	52920
Slope	2643	1055	35514

Fig.1.2: Linearity plot for HCTZ, Amlodipine, Valsartan







Precision: For the precision study, repeatability study was carried out for short time interval under the same chromatographic conditions. For the intermediate precision study, repeatability study was carried out in different day under the same chromatographic conditions. The standard was injected in six replicate. The peak area for all the six replicate was recorded. The mean and % relative standard deviation (%RSD) was calculated. From the data obtained the developed RP-HPLC method was found to be precise. The results were shown in Table-3 and ID Precision in Table-4.

Table-3: Precision results for HCTZ, Amlodipine, Valsartan

S.No.	HCTZ	Amlodipine	Valsartan
1	248354	120270	3535290
2	248360	120373	3535304
3	248224	120456	3536204
4	248150	120644	3539742
5	248597	120457	3535938
6	247346	120482	3533717
Average	248172	120447	3536033
Standard deviation	432.33	124.11	2012.08
% RSD	0.17	0.10	0.06

Table-4: ID Precision results for HCTZ, Amlodipine, Valsartan

S.No.	HCTZ	Amlodipine	Valsartan
1	249409	120652	3546756
2	248829	120582	3536221
3	248321	120713	3542476
4	249202	120536	3530457
5	248638	120461	3535846
6	248757	120403	3533041
Average	248859	120558	3537466
Standard deviation	392.36	116.06	6071.00
% RSD	0.16	0.10	0.17

Method precision: Twenty tablets of Exforge HCT were weighed to get the average weight and then ground. An amount of powder equivalent to 25 mg, 10mg, 320 mg of HCTZ and Amlodipine and Valsartan Tablet powder respectively into a 250 ml volumetric flask individually add methanol and sonicate to dissolve it completely and make volume up to the mark with the same solvent.Further pipette 5.0 ml of above stock solution into a 20ml volumetric flask and dilute up to the mark with diluent. The above solution was injected for 6 times. The mean and % relative standard deviation (%RSD) was calculated. From the data obtained the developed RP-HPLC method was found to be precise. The results were shown in Table-5.

Table-5: Method Precision results for HCTZ, Amlodipine, Valsartan

S.No.	HCTZ	Amlodipine	Valsartan
1.	102.01	99.36	98.46
2.	102.03	99.31	98.50
3.	102.03	99.13	98.50
4.	99.76	99.14	98.45
5.	102.04	98.85	98.46
6.	101.12	98.92	98.21
Average	101.5	99.1	98.4
Standard deviation	0.85	0.20	0.11
% RSD	0.84	0.21	0.11

Accuracy: The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed tablet sample. Percent recovery was calculated by comparing the area with preanalysed sample. For both the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 50%, 100%, 150% level and the percentage recovery was calculated by subtracting the total area from preanalysed sample area. The results were shown in Table-6.

Table 6: Accuracy Results For HCTZ, Amlodipine, Valsartan

Analyte	% Level	Nominal Value (mg)	Found (mg)	% Recovery	Mean % Recovery
HCTZ	50%	12.5	12.45	99.56	100.12
	100%	25	25.10	100.38	
	150%	37.5	37.65	100.4	
Amlodipine	50%	5	4.92	98.39	100.43
	100%	10	10.21	102.08	
	150%	15	15.12	100.83	
Valsartan	50%	16	16.20	101.24	99.88
	100%	32	32.11	100.33	
	150%	48	47.04	98.07	

Robustness: Robustness of the method was checked by making slight deliberate changes in chromatographic conditions like flow rate (0.2ml/min). It was observed that there were no marked changes in system suitability parameters, which demonstrated that the developed RP-HPLC method is robust.

Table 7: Robustness Results for HCTZ, Amlodipine, Valsartan

	HCTZ			Amlodipine			Valsartan		
	RT	USP Plate count	USP Tailing	RT	USP Plate count	USP Tailing	RT	USP Plate count	USP Tailing
Flow 1	1.7	2899	1.49	3.6	14531	1.16	6.0	21704	1.07
Flow 2	1.45	2893	1.54	3.06	11391	1.1	5.3	23931	1.09

Flow 1- 0.8ml/min, Flow 2- 1.2ml/min

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine HCTZ, Amlodipine, Valsartan in their tablet dosage form. The result obtained for HCTZ, Amlodipine, Valsartan was comparable with the corresponding labeled amounts and they were shown in Table-8.

Table 8: Assay Results

	Label claim	% Assay
HCTZ	25 mg	101.87
Amlodipine	10 mg	98.17
valsartan	320mg	98.01

Degradation studies: The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Exforge using the proposed method.

Preparation of Stock Solution: An amount of tablet powder equivalent to 25 mg, 10mg, 320mg of HCTZ and Amlodipine and Valsartan Tablet powder respectively into a 250 ml volumetric flask individually add methanol and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

ACID degradation condition: Pipette 5ml of the above stock solution into a 20ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 90ºC for 1 hour and then neutralized with 0.1 N NaOH and make up to 20ml with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

ALKALI degradation condition: Pipette 5 ml of the above stock solution into a 20ml volumetric flask and add 3 ml of 0.1N NaOH was added in 20 ml of volumetric flask. Then, the volumetric flask was kept at 90ºC for 2 hour and then neutralized with 0.1N HCl and make up to 20ml with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

Thermal induced degradation: The sample was taken in petridish and kept in Hot air oven at 110^0 C for 24 hours. Then the sample was taken and diluted with diluents to prepare 25 ug/ml,10 ug/ml,320 ug/ml of HCTZ, Amlodipine, Valsartan and injected into HPLC and analysed.

Oxidative degradation: Pipette 5 ml of the above stock solution into a 20ml volumetric flask 3 ml of 3% w/v of hydrogen peroxide added in 20 ml of volumetric flask and the volume was made up to the mark with diluent . The volumetric flask was then kept at room temperature for 1hr min. Filter the solution with 0.45 microns syringe filters and place in vials.

Results :Degradation Data

Conditions	%Total Degradation
Acid	22%
Base	17%
Peroxide	20%
Thermal	6%

CONCLUSION

In the present work a new, accurate, precise and robust HPLC method was developed and validated for estimation of HCTZ, Amlodipine, Valsartan in pharmaceutical dosage form in accordance with the ICH parameters. The method gives good resolution between both the compounds with a short analysis time (10min). Linearity is observed in the concentration range from 12.5-37.5 µg/ml for HCTZ, 5-15 µg/ml for Amlodipine, 160-480 µg/ml for Valsartan drugs at 251 nm. The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be useful for the routine analysis of the HCTZ, Amlodipine, Valsartan in combined dosage form without any interference of excipients.

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