IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 3 Issue 6, June 2016
ISSN (Online) 2348 – 7968 | Impact Factor (2015) - 4.332
www.ijiset.com

Development and Validation Of A Simple, Quick And Rugged Isocratic HPLC-UV Method for the Detection of Valsartan and Hydrochlorothiazide in Their Combined Pharmaceutical Formulations

Babiker Y. Abdulkhair [a,b,]*, Mohamad A. Abdulaziz [a].

[a] Chemistry Departemt, College of Science, Sudan University For Science and Technology, Khartoum, Sudan.

Key words: HPLC-UV; Isocratic; Method validation; Valsartan; Hydrochlorothiazide.

* Corresponding author: Cell phone: +966542058188; +966538964177. E-mail address: babiker35.by@gmail.com

Abstract

A simple, isocratic RP HPLC-UV method was developed for the simultaneous determination of the antihypertensive combination of valsartan (VAL) and hydrochlorothiazide (HCTZ) in bulk and their formulations. The challenge was to develop a simple, accurate and robust isocratic method. An excellent separation obtained by Phenyl-hexyl column (150 mm × 4.6 mm, 3µm). mobile phase was a formic acid solution (1% v/v) : acetonitrile in a 25:75 ratio, flow rate was 0.8 ml/min. Both active ingredients were detected at 275 nm, injection volume was 20 µl and the analysis temperature was 25^{o}C (ambient temperature). Resolution = 7.59, retention times was 2.3 min and 3.5 min for HCTZ and VAL respectively. The proposed method was tested for system suitability, linearity, range, precision, accuracy, specificity, robustness, detection and quantification limits. The linearity range was 0.17-10 µg/ml HCTZ and 1.6-20 µg/ml VAL. The correlation coefficient of the regression line was 1.000 for both components. Method robustness was tested under nine different conditions using samples with a known content. For HCTZ, the mean of the nine assays was 100.7% and the RSD was 0.48%. For VAL, the mean of the nine assays was 100.31% and the RSD was 0.26%. The results show that this is a simple method that can be applied to the analysis of combined antihypertensive drug tablets with satisfactory degrees of accuracy and precision. Due to the selected optimized conditions, this method can be used with the minimum requirements of an isocratic HPLC system.

1. Introduction

Hypertension has been recognized as an important risk factor for cardiovascular disease and is a leading risk factor for mortality [1] As it has become evident that monotherapy cannot achieve BP goals in the majority of patients, particularly high-risk patients who need stricter BP control, multiple studies have looked into the beneficial effects of initial combination therapy in patients with hypertension [2]. Hydrochlorothiazide (HCTZ) (Fig. 1) is one of the oldest thiazide diuretics, often prescribed in combination with other antihypertensive drugs such as beta blockers, angiotensin-converting enzyme inhibitors, or angiotensin II receptor blockers. Valsartan (VAL) (Fig. 2) is an antihypertensive drug belonging to the family of angiotensin II receptor antagonists [3]. Recently, a combination of hydrochlorothiazide and valsartan has been applied for treatment and management of hypertension. It was found that the use of this combination was generally more effective in reducing blood pressure and providing overall blood pressure control than dual combination therapies regardless of age, race, gender, or hypertension severity [4,5]. Amlodipine besylate and VAL can be determined simultaneously in their binary combination using several methodologies such as UV spectrophotometry [6-12], spectrofluorimetry [13-15] and HPLC methods with UV detection [1 6-20]. The HPLC method is quick (had short analysis time), easy (buffer is formic acid solution (1% v/v), no pH adjustment required), simple (0.8 ml/min isocratic elution), accurate and rugged. This assay method has been validated according to ICH guidelines [21].

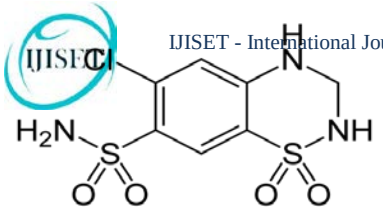

Fig. 1: Chemical structure of hydrochlorothiazide

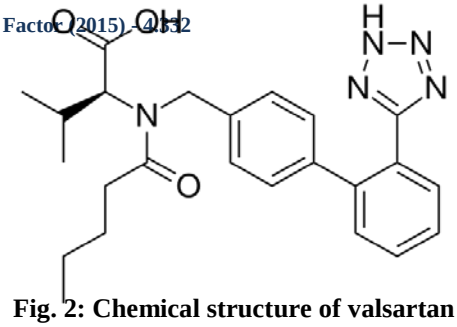

Fig. 2: Chemical structure of valsartan

2. Experimental

2.1. Chemicals

Working standards of VAL and HCTZ and excipients were supplied from Blue Nile Pharmaceuticals. Acetonitrile and formic acid were HPLC grade (Scharlau Spain). HPLC grade water was used, and tablets labeled 160 mg VAL and 12.5 mg HCTZ were collected from local a market in Riyadh, KSA.

2.2. Instrumentation

The HPLC-UV system consisted of a Shimadzu LC-2010A HT series apparatus (Shimadzu Corporation, Tokyo, Japan) with a quaternary pump, online degasser, UV detector, column oven and auto sampler. This system was connected to a computer loaded with LC-Solutions software. A Phenyl-Hexyl column (150 mm x 4.6 mm, I.D. 3 µm) was used. DAD, Shimadzu Corporation, Tokyo - Japan, Prominence, - Quaternary pump - Sr. No.L2010482018

2.3. Methods

2.3.1. Standard stock solution

To prepare stock solutions, 0.16 g of VAL and 0. 0125 g of HCTZ were weighed accurately and transferred quantitatively to the same 100 ml volumetric flask. The flask was half-filled with the mobile phase and sonicated for 10 minutes, cooled to room temperature, then the volume was completed to the mark with the same solvent.

2.3.2. Standard solution

Subsequent dilutions were made from the stock solution with the mobile phase to make solutions with 6.25 µg/ml of HCTZ and 40 µg/ml of VAL. The resulting solution was filtered through a 0.45 µm membrane nylon filter.

2.3.3. Assay preparation

Twenty tablets were weighed, transferred to a mortar and ground. The average weight of the tablet was transferred to 100ml volumetric flask, then half filled with mobile phase and sonicated for 10 minutes, cooled to room temperature then the volume was completed to the mark with the same solvent. Subsequent dilutions were made in the mobile phase in the same manner as the standard to achieve the target concentration. The resulting solution was filtered through a 0.45 µm membrane nylon filter. The recovered concentration was calculated by comparing the analyte response of the sample with that of the standard.

2.4. Optimized chromatographic conditions

The mobile phase was composed of 1%v/v formic acid solution and acetonitrile 25:75, using isocratic elution with a flow rate of 0.8 ml/min. The injection volume was 20 µl, using a Phenyl-Hexyl column (150 mm x 4.6 mm, 3 µm). The eluents were monitored at 275 nm and the method was optimized at 25oC.

3.Results and discussion

3.1. Validation of the developed and optimized method

The validation of the developed method was done as per ICH guidelines which include system suitability, linearity, specificity, accuracy, interday precision, intraday precision and robustness.

3.1.1. System suitability

The system suitability test is an integral part of the analytical method. For this, a mixed standard solution (target concentration) was injected six times. Parameters such as RSD% for the peak area, retention time, resolution and theoretical plates of the peaks were calculated. The results for HCTZ and VAL are shown in Table 1 and Table 2, respectively.

	Retention time	Area	Theoretical plates	Tailing factor	Resolution
STD 1	4.512	619051	7712	1.293	12.02
STD 2	4.511	617597	7713	1.292	12.035
STD 3	4.511	619530	7763	1.294	12.04
STD 4	4.511	619240	7718	1.295	11.993
STD 5	4.515	619382	7758	1.295	12.019
STD 6	4.512	618804	7735	1.294	12.019
Avarage	4.512	618934	7733.166667	1.293833333	12.021
STDEV	0.001549193	702.526	22.7808399	0.001169045	0.01643168
RSD	0.034334959	0.11351	0.294586175	0.090355161	0.13669143

Table .1: System suitability parameters for HCTZ

	Retention time	Area	Theoretical plates	Tailing factor	Resolution
STD 1	2.302	562357	3219	1.346	12.02
STD 2	2.301	562920	3238	1.344	12.035
STD 3	2.302	562108	3222	1.347	12.04
STD 4	2.301	562314	3177	1.351	11.993
STD 5	2.301	563103	3178	1.352	12.019
STD 6	2.301	561654	3199	1.343	12.019
Avarage	2.301333333	562409.3333	3205.5	1.347166667	12.021
STDEV	0.000516398	531.9983709	24.98599608	0.003656045	0.01643168
RSD	0.022439069	0.094592735	0.779472659	0.271387744	0.13669143

Table. 2: System suitability parameters for VAL

3.1.2. Selectivity

The standard solution was prepared and injected (Fig. 3). The sample and placebo solutions were prepared by taking the weight of placebo equivalent to its weight in the test preparation. Based on the chromatograms of the sample (Fig. 4) and placebo (Fig. 5), the placebo solutions showed no peaks at the retention time of the HCTZ and VAL peaks. This indicates that the excipients used in the formulation did not interfere in the estimation of the active ingredients in the tablets. Also, based on Fig. 3 and Fig. 4, the system suitability parameters in the sample chromatogram were almost equal to those of the standard chromatogram (i.e. the excipients in the sample did not affect separation).

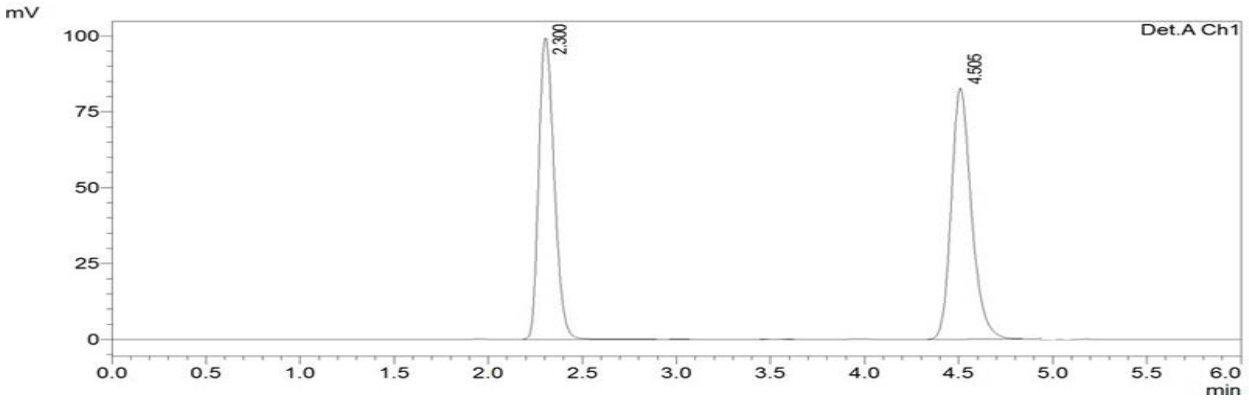

Fig. 3: Chromatogram of the mixed standard solution under the optimized conditions

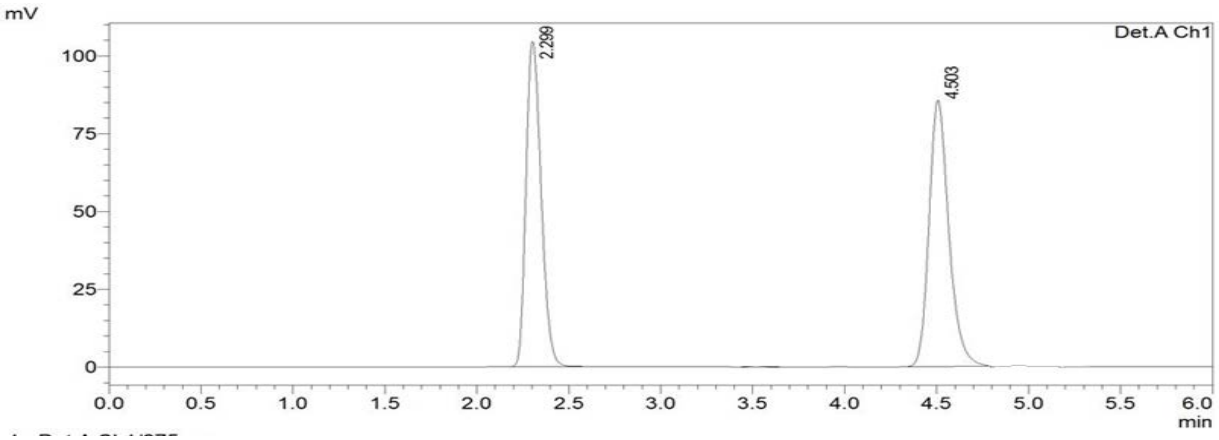

Fig. 4: Chromatogram of a sample solution under the optimized conditions

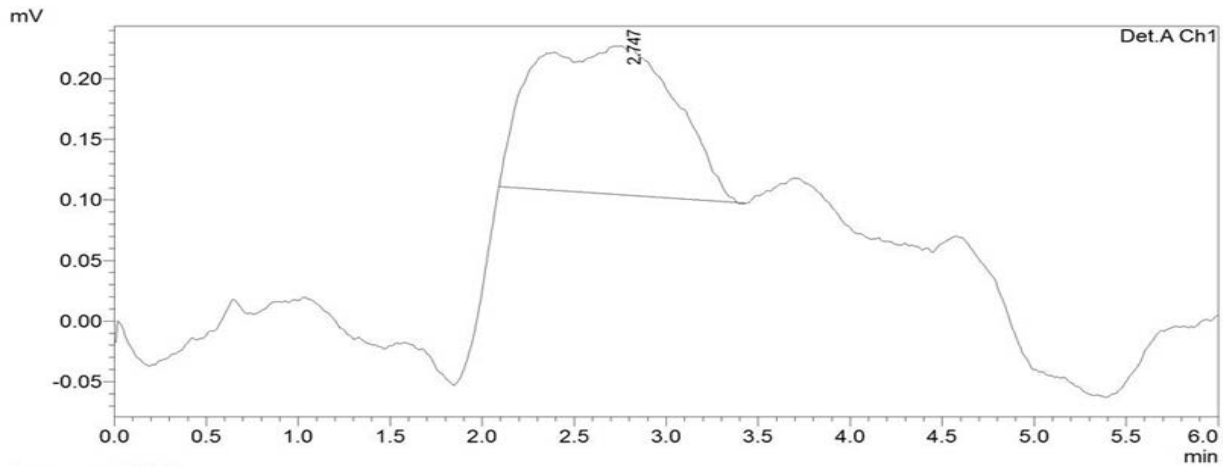

Fig. 5: Chromatogram of a placebo solution under the optimized conditions

3.1.3. Linearity

Seven concentrations were prepared, ranging from 40% to 160% of the target analyte concentrations; typically, 2.5, 3.75, 5, 6.25,7.5 ,8.75 and 10 µg/ml HCTZ solutions and 5,7.5,10,12.5,15,17.5 and 20µg/ml valsartan solutions were prepared in the mobile phase as mixed standards. Each standard mixture was injected in triplicate and the mean value of the peak area was used for the calibration curve. The calibration graph was obtained using XL-STAT 2015. The linear regression plots for HCTZ (Fig. 6) and VAL (Fig. 7) show that the regression equations were **Area=9735.87+91787.13*µg/ml** and **Area=19.083+15534.06*µg/ml**, respectively. The regression coefficient values (R^2) were found to be 1.000 for both analytes, indicating an excellent degree of linearity.

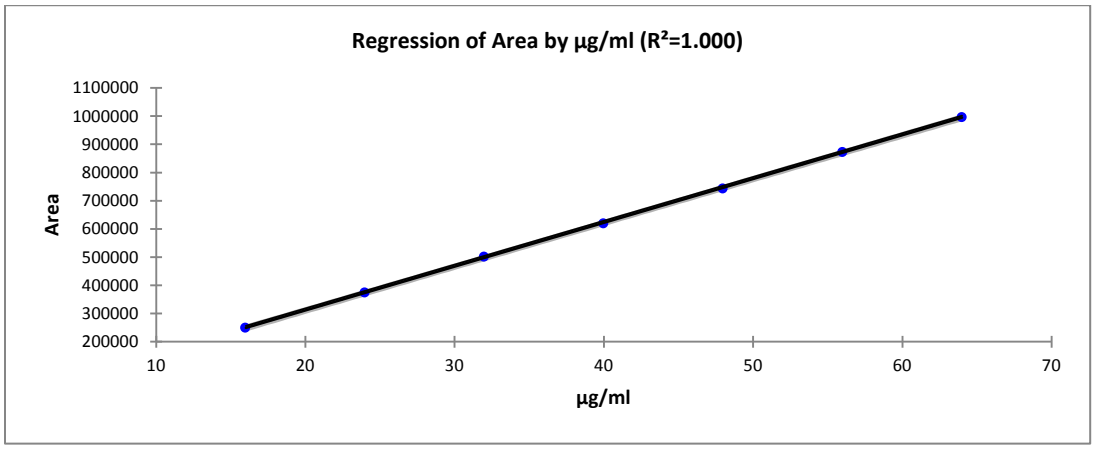

Fig. 6: XL- STAT 2015 plot of (µg/ml) Vs (peak area) - hydrochlorothiazide

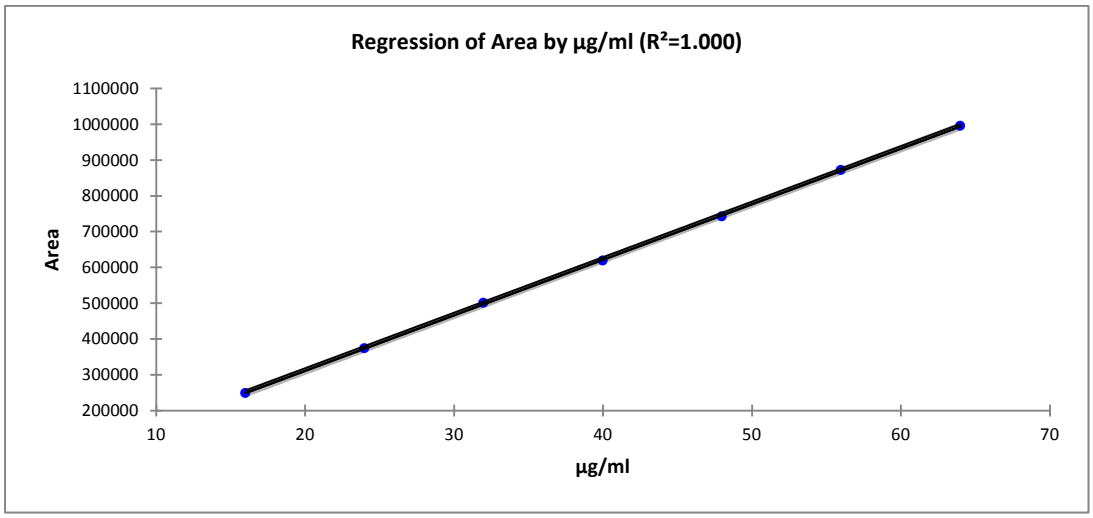

Fig. 7: XL- STAT 2015 plot of (µg/ml) Vs (peak area) - Valsartan

3.1.4. Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated from linearity data according to ICH [22]: LOD = 3.3* (SD/S) and LOQ = 10 * (SD/S). The LOD was found to be 0.056 µg/ml and 0.5276 µg/ml for HCTZ and VAL, respectively, while the LOQ values were 0.17 µg/ml and 1.599 µg/ml, respectively.

3.1.5. Accuracy

Seven 100 ml volumetric flasks were labeled, and the placebo equivalent to a tablet's weight was transferred to a different flask. The volume of the mixed standard stock solution required to produce 40%, 60%, 80%, 100%, 120%, 140% and 160% of the target concentration of both HCTZ and VAL was added to the flasks. The flasks were half-filled with the mobile phase, sonicated for 10 minutes, cooled to room temperature, then completed to the mark with the same solvent. Subsequent dilutions were made with the mobile phase in the same manner as the standard preparation. The assay was performed on the seven solutions. The recovery percentage for HCTZ and VAL was found to be within the acceptance criteria, i.e. the mean, standard deviations and relative standard deviations of the recovery percentage of the seven different concentrations were found to be within acceptance limits (Table 3).

Amount added (%)	(HCTZ)		(VAL)	
	Recovery	Recovery%	Recovery	Recovery%
40	40.159	100.3974	40.2135	100.5337
60	60.046	100.0773	60.1246	100.2077
80	80.023	100.0293	80.446	100.5572
100	100.87	100.8663	100.26	100.2629
120	120	100.0029	119.82	99.84749
140	141.24	100.8855	140.8129	100.5806
160	161.25	100.7796	159.92	99.95158
avg		100.44		100.21
STDEV		0.62408		0.5184
RSD		0.62132		0.51729

Table .3: Accuracy results for HCTZ and VAL

3.1.6. Precision

3.1.6.1. Intraday precision

Three 50 ml volumetric flasks were labeled, and the placebo equivalent to one tablet was transferred to each flask. The volume of the standard stock solution required to produce 80%, 100% and 120% of the tablet content of both hydrochlorothiazide and valsartan was added. The flasks were half-filled with the mobile phase, sonicated for 10 minutes, cooled to room temperature and completed to the mark with the same solvent. Subsequent dilutions were made with the mobile phase in the same manner as the standard preparation. The assay was performed on these solutions five times in one day; each solution was injected three times for each assay. The means, standard deviations and relative standard deviations of the assays were calculated; the method's interday precision was found to be within the permissible limits. The results are shown in Table 4.

Table .4: Results for intraday precision

Trial No.	(HCTZ)			(VAL)		
	80%	100%	120%	80%	100%	120%
Assay 1	100.52	100.46	100.22	100.48	100.41	99.607
Assay 2	99.884	100.75	99.997	100.36	100.4	100.04
Assay 3	100.32	100.67	100.3	100.13	100.48	99.9
Assay 4	100.27	100.81	100.26	100.42	100.5	99.946
Assay 5	100.19	100.46	100.41	100.52	100.8	100.09
avg	100.24	100.63	100.24	100.38	100.52	99.916

STDEV	0.2324	0.1622	0.152	0.1524	0.1631	0.1882
RSD	0.2319	0.1612	0.1517	0.1518	0.1622	0.1884

3.1.6.2. Intraday precision

Three 50 ml volumetric flasks were labeled, and the placebo equivalent to one tablet was transferred to each flask. The volume of standard stock solution required to produce 80%, 100% and 120% of the tablet content of both hydrochlorothiazide and valsartan was added. The flasks were half-filled with the mobile phase, sonicated for 10 minutes, cooled to room temperature and completed to the mark with the same solvent. Subsequent dilutions were made with the mobile phase in the same manner as the standard preparation. The assay was performed on these solutions three times on three different days. The solutions were injected three times for each assay. The means, standard deviations and relative standard deviations of the assays were calculated; the method's intraday precision was found to be with the permissible limits. The results are shown in Table 5.

Table .5: Results for interday precision

	(HCTZ)			(VAL)		
	80%	100%	120%	80%	100%	120%
Day 1	100.52	100.46	100.22	100.48	100.41	99.607
Day 2	99.863	100.89	100.44	100.38	100.34	100.01
Day 3	100.15	101.28	100.54	100.61	100.82	100.34
avg	100.18	100.88	100.4	100.49	100.52	99.986
STDEV	0.3308	0.4092	0.1642	0.117	0.2539	0.3665
RSD	0.3302	0.4057	0.1635	0.1164	0.2526	0.3665

3.1.7 Robustness

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. An assay was performed with the following variations: optimum conditions, 5ºC higher or lower, 5% more or less organic solvent in the mobile phase, 5% increase or decrease in the flow rate of the mobile phase, and detection 3 nm above or below the detection wavelength. The results were collected and subjected to statistical treatments. The means, standard deviations and relative standard deviations for the assay under all studied conditions are shown in Table 6.

Table. 6: Robustness of the method

Condition	Recovery%	
	(HCTZ)	(VAL)
Optimized conditions	100.05222	99.990844
More 5 degree Celsius	100.63584	100.78699
less 5 degree Celsius	100.73757	100.07426
5% More flow rate	100.30306	100.2931
5% less flow rate	100.80281	100.15821
5% more Organic solvent	101.71063	100.60529
5% less Organic solvent	100.61784	100.28913
More 3 nm	100.61317	100.53662
Less 3 nm	100.22635	100.17157
avg	100.69428	100.31398
STDEV	0.4760809	0.2657388
RSD	0.4727984	0.2649071

4. Conclusions

In this study, a simple, specific and reliable isocratic elution HPLC-UV procedure was developed to assess HCTZ and VAL in their pharmaceutical combination. The most important feature in the proposed method is its simplicity, as this method can be used with the minimum requirements of an isocratic HPLC system (one pump), UV detection at the same wavelength (the most common detector and no gradient program required). The method was optimized to be used at ambient temperature (no column oven required). Moreover, the method is economic (flow rate 0.8 ml/min for 5 min per injection), the buffer solution is easy to prepare (1% formic acid solution without pH adjustment), and the method passed all tests of robustness. To the best of our knowledge, no simpler method has been reported for an assay for this drug mixture. This method is applicable even with conventional HPLC systems, because of that its recommended quality control purposes.

References

[1]E zzati M, Lopez AD, Rodgers A, Vander HS, Murray CJ. Selected major risk factors and global and regional burden of disease. Lancet 2002;360:1347-1360.

[2] Salahuddin A, Mushtaq M, Materson BJ American Society of Hypertension, Journal of the American Society of Hypertension 2013;7(5):401-407
[3] Thurmann PA. Expert Opin. Pharmacother. 2000;1(2):337
[4] Chrysant SG.. Single-pill triple-combination therapy: an alternative to multiple-drug treatment of hypertension. Postgrad. Med. 2011;123(6):21-31.
[5] Calhoun DA, Lacourciere Y, Chiang YT, Glazer RD. Triple antihypertensive therapy with amlodipine, valsartan, and hydrochlorothiazide. Hypertension 2009;54(1):32-39.
[6] Sayyed ZM, Shinde SA, Chaware VJ, Chaudhari BP. Development and validation of UV- spectrophotometric method for simultaneous estimation of spironolactone and hydrochlorothiazide in pharmaceutical formulation. J. Pharm. Sci. Bioscientific. Res. 2015;5(6):590-593.
[7] Gupta KR, Mahapatra AD, Wadodkar AR, Wadodkar SG.. Simultaneous UV spectrophotometric determination of valsartan and amlodipine in tablet. Int. J. Chem. Tech. Res. 2010;2(1):551-556.
[8] Redasani VK, Patel PV, Surana SJ. Spectrophotometric method for simultaneous estimation of Valsartan and Hydrochlorothiazide in combined tablet dosage form, Der Pharmacia Sinica 2011;2(3):123-130.
[9] Patel NR, Patel SK. Int. J. of Pharm. Life Sci. 2012;3(7):1828-1832
[10] Rezk MR, El Remali NM, El Bayoumi Abdel Aleem AA. Simultaneous determination of valsartan and hydrochlorothiazide in their pharmaceutical formulations, Der Pharma Chemica, 2012;4(1):529-537.
[11] Chaudhary A, Patel RK, Chaudhary SA, Gadhvi KV. Estimation of valsartan and hydrochlorothiazide in pharmaceutical dosage forms by absorption ratio method. Int. J. Appl. Biol. Pharm. Technol. 2010;1(2):455- 464.
[12] Kul D, Dogan-Topal B, Kutucu T, Uslu B, Ozkan SA. High-performance liquid chromatographic and first derivative of the ratio spectrophotometric determination of amlodipine and valsartan in their binary mixtures. J. AOAC Int. 2010;93(3):882-890.
[13] Anumolu PD, Sunitha G, Bagirath R, Vani PS, Archana G, Zero-crossing point derivative simultaneous spectrofluorimetric method for quantification of nebivolol hydrochloride and valsartan combination in tablets, Int. J. Pharm. Sci. Rev. Res. 2014;27(2):164-168.
[14] Abd-El-Fattah Mohammed T. Native and synchronous spectrophotometric method for simultaneous determination of amlodipine besylate and valsartan combination in tablets. Asian J. Sci. Technol. 2015;6(8):1690-1698.
[15] El-Shaboury SR, Hussein SA, Mohamed NA, El-Sutohy MM. Spectrofluorimetric method for determination of some angiotensin II receptor antagonists, J. Pharm. Anal. 2012;2(1):12-18.
[16] Shaalan RA. et al., alidated stability-indicating HPLC-DAD method of analysis for the antihypertensive triple mixture of amlodipine besylate, valsartan and hydrochlorothiazide in their tablets. Arab. J. Chem. 2013. http://dx.doi.org/10.1016/j.arabjc.2013.04.012

[17] H Li, Wang Y, Y Jiang, Tang Y, Wang J, Zhao L, Gu J. J. Chromatogr B., 2007;852:436-442
[18] El-Gizawy SM, Abdelmageed OH, Omar MA, Deryea SM, Abdel-Megied AM. Development and validation of HPLC method for simultaneous determination of amlodipine, valsartan, hydrochlorothiazide in dosage form and spiked human plasma. Am. J. Anal. Chem. 2012;3:422-430.
[19] Liu F, Zhang J, Xu Y, Gao S, Guo Q. Simultaneous determination of hydrochlorothiazide and valsartan in human plasma by liquid chromatography/tandem mass spectrometry. Anal. Lett. 2008;41(8);1348-1365.
[20] Lakshmi KS, Lakshmi S. Simultaneous spectrophotometric determination of valsartan and hydrochlorothiazide by H-point standard addition method and partial least squares regression, Acta Pharm. 2011;61:37-50.
[21] International conference on harmonization of technical requirements for registration of pharmaceuticals for human use - Q2(R1) - Parent Guideline dated 27 October 1994.