HTTPS://DOI.ORG/10.33472/AFJBS.6.5.2024.6147-6158

African Journal of Biological Sciences

Research Paper Open Access

Separation And Quantification Of Azelnidipine And Chlorthalidone By Optimized Rapid RP-HPLC Method In Fixed Dose Combination

Dr. Manasi J. Wagdarikar[1*,] Dr. Anagha M. Joshi[2] , Yogita A. Nimbalkar[3]

[1*,2,3]Department of Pharmaceutical Chemistry, SCES's Indira College of Pharmacy, Pune 411033, Maharashtra, India.

***Corresponding Author**: Dr. Manasi J. Wagdarikar
*Department of Pharmaceutical Chemistry, SCES's Indira College of Pharmacy, Pune 411033, Maharashtra, India

ABSTRACT
Chlorothalidone and Azelnidipine are used as antihypertensive drugs alone or in combination form. A simple, precise, and cost-effective RP- HPLC method was developed for Chlorothalidone and Azelnidipine in bulk dosage form. Chromatographic separation was done by Agilent Zorbax Bonus RP (250 mm x 4.6 mm, 5µm) column. To achieve better results, the mobile phase composition was changed to Acetonitrile and 0.1% Perchloric acid in the ratio of 55:50 %v/v at 1.0 ml/min flowrate at 270 nm. Runtime was 10 minutes and the retention times for Chlorthalidone and Azelnidipine were found to be 3.10 and 6.62 minutes, respectively. The method is validated for specificity, Assay, Repeatability, system suitability, linearity, range, accuracy, LOD, LOQ.

Keywords: Chlorothalidone, Azelnidipine, RP- HPLC, Validation, Quantification, fixed dose combination

Introduction

Combination therapy is frequently used to treat hypertension in order to increase effectiveness and address a variety of pathophysiological processes related to blood pressure regulation. Because of their complimentary modes of action, azelnidipine, a dihydropyridine calcium channel blocker (CCB), and chlorthalidone, a thiazide-like diuretic, constitute a strong combination. Azelnidipine works by preventing calcium from entering vascular smooth muscle cells, which causes vasodilation and a drop in blood pressure.[1-5] Contrarily, chlorthalidone increases diuresis by preventing the kidneys' distal convoluted tubules from reabsorbing sodium, which lowers blood pressure and plasma volume.[6-8] Azelnidipine and chlorthalidone together have several benefits. First, it treats volume overload in addition to peripheral vascular resistance, which results in a more thorough antihypertensive action. Second, by combining drugs with distinct mechanisms of action, side effects that may result from using greater doses of separate agents of medicine might possibly be minimized by using lower doses of each drug.[9-12]

Clinical research has shown that azelnidipine-chlorthalidone combination therapy is safe and effective at reducing blood pressure. For instance, in individuals with essential hypertension, azelnidipine monotherapy and combination therapy with chlorthalidone were examined for efficacy in a randomized controlled trial. According to the study, combination medication had a better tolerability profile and considerably reduced both systolic and diastolic blood pressure when compared to monotherapy. [13-17]

Structures of both the drugs are given below.[18-19]

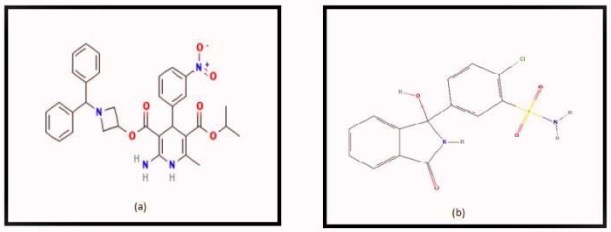


Fig. 1. Structure of a) Azelnidipine and b) Chlorthalidone

Reverse-phase high-performance liquid chromatography (RP-HPLC) is a crucial analytical technique widely employed for the determination of pharmaceutical compounds due to its high sensitivity, selectivity, and reproducibility [20-23]. The published data gives few RP-HPLC methods for simultaneous estimation of Chlorthalidone and Azelnidipine.[24] In present study, new RP-HPLC method is developed and validated with the advantage of improved retention times of both the drugs in combination.

Materials and Methods

Standard Azelnidipine and Chlorthalidone were procured as gift samples from Aadhaar Life Sciences Pvt. Ltd., Solapur, India. Acetonitrile, Water and Perchloric acid of AR grade from Merck Life Science Pvt. Ltd, was used. A commercial dosage from UNIAZ CH 8/6.25 was purchased from the local market. Agilent 1260 infinity II was used for HPLC method development.

I. Preparation of Standard Stock Solution (SSS-I)

Standard Stock Solution-I (SSS-I):

Initially Prepare a Standard Stock Solution (SSS-I) of Chlorthalidone by adding 12.5 mg in 10 ml volumetric flask & add 5 ml acetonitrile, mix for 2 minutes, and make the volume to 10 ml with diluent. (Conc. of Chlorthalidone = 1250 µg/ml).

Standard Stock Solution-II (SSS-II):

Then prepare a Standard Stock Solution (SSS-II) of Azelnidipine by adding 8 mg in 10 ml volumetric flask & add 5 ml Acetonitrile, mix for 2 minutes, and make the volume to 10 ml with Methanol. (Conc. of Azelnidipine = 800 µg/ml). Then add 0.5 ml of SSS-I & 1.0 ml SSS-II in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Chlorthalidone = 62.5 µg/ml & Azelnidipine = 80 µg/ml)

II. Tablet Sample Preparation:

Tablet powder equivalent to 6.25 mg chlorthalidone and 8 mg of azelnidipine was weighed and mixed with Acetonitrile and sonicated for 5 minutes. (Stock conc of Chlorthalidone = 625 µg/ml & Azelnidipine = 800 µg/ml). 1 ml of above solution was further diluted to 10 ml with diluent. (Conc of Chlorthalidone = 62.5 µg/ml & Azelnidipine = 80 µg/ml)

III. Selection of Analytical Wavelength

To investigate the appropriate wavelength for determination of Azelnidipine and Chlorthalidone solution in the mobile phase were scanned HPLC- DAD in the range of 190-400nm.

IV. Selection of column (stationary phase)

To get well resolved, symmetric peak with highest number of theoretical plates the solution of the Azelnidipine and Chlorthalidone were analyzed using Zorbax Bonus RP column as a stationary phase.

V. Chromatographic Conditions

Analytical Column: Zorbax Bonus RP (250 x 4.6 mm, 5µ)
Mobile Phase: 0.1% Perchloric acid: Acetonitrile (50:50, % v/v)
Flow Rate: 1 ml/min
Injection Volume: 10 µl
Detection Wavelength: 250 nm

VI. Preparation of Mobile Phase

Mobile phase was prepared by mixing 500 ml of 0.1 % Perchloric acid and 500 ml of Acetonitrile and filtered through 0.45µm nylon filter using vacuum pump and ultra sonicate for 30 min for degassing.

VII. Preparation of 0.1 % Perchloric Acid

1 ml of Perchloric Acid was added to 500 ml of Type I water in a 1000 ml beaker and mixed. Volume was made up to the mark using Type I water.

Results and Discussion

1. HPLC Method Development

The diluent selected was Acetonitrile and 0.1% Perchloric Acid (50:50, % v/v). Therefore, working standard solutions were prepared and stored at room temperature. The Stationary phase selected for the analysis was Agilent Zorbax Bonus RP (250 x 4.6 mm, 5µ) which helped in acquiring moderate retention of Chlorthalidone and Azelnidipine.

The Method Development of Chlorthalidone and Azelnidipine was initiated by taking mobile phase Acetonitrile and 0.1% Perchloric acid in the ratio of 50:50 %v/v at flowrate 1 ml/min by using Agilent Zorbax Bonus RP (250 x 4.6 mm, 5µ) at 250 nm. The Runtime was 10 min and the retention times for Chlorthalidone and Azelnidipine was found to be 3.09 and 6.61 minutes, respectively. Both the peaks were well resolved. Theoretical plates of both the peaks was found to be above 2000 and asymmetry less than 2.0.

To achieve better results, the mobile phase composition was changed to Acetonitrile and 0.1% Perchloric acid in the ratio of 55:45 %v/v at 1.0 ml/min flowrate at 250 nm. The Runtime was 10 minutes and the retention times for Chlorthalidone and Azelnidipine was found to be 2.81 and 3.65 minutes, respectively. Both the peaks were well resolved, but the resolution was found to be less than the Trial 1. Theoretical plates of both the peaks was found to be above 2000 and asymmetry less than 2.0.

To increase the resolution, the mobile phase composition was changed to Acetonitrile and 0.1% Perchloric acid in the ratio of 52.5:47.5 %v/v at 1.0 ml/min flowrate at 250 nm. The Runtime was 10 minutes and the retention times for Chlorthalidone and Azelnidipine was found to be 2.89 and 4.37 minutes, respectively. Both the peaks were well resolved, but the resolution was found to be less than the Trial 1 and more than Trial 2. Theoretical plates of both the peaks was found to be above 2000 and asymmetry less than 2.0.

To check the effect of stationary phase, Regular C18 column i.e. Phenomenex Kinetex XB-C18 was used with similar chromatographic conditions as that of Trial 3. Both the peaks got eluted within 5 minutes. The Chlorthalidone was found to be 1.55 minutes and 4.19 minutes for Azelnidipine. As the Chlorthalidone eluted faster which means there was no retention observed. The theoretical plates were found to be above 2000 for both the peaks but were less than previous Trials. Hence, Agilent Zorbax Bonus RP (250 x 4.6 mm, 5µ) was found to be the suitable column for simultaneous determination of Chlorthalidone and Azelnidipine.The results for different method development trials are given in table 1.

Table 1: Method Development Trials

Trial No.	Mobile Phase	Ratio	Diluent	Column	Wave-length	Chlorthalidone				Azelnidipine			
						RT	TP	Aysmm-etry	Resolu-tion	RT	TP	Aysmm-et ry	Resolu-ti on
1	ACN : 0.1% PCA	50-50	50 ACN: 50 0.1% PCA	Agilent Zorbax Bonus RP (250 x 4.6mm, 5µ)	250	3.09	12849	1.07	0.00	6.61	5387	0.86	14.98
2	ACN : 0.1% PCA	55-45	50 ACN: 50 0.1% PCA	Agilent Zorbax Bonus RP (250 x 4.6mm, 5µ)	250	2.81	12746	1.01	0.00	3.65	6862	0.89	6.10
3	ACN : 0.1% PCA	52.5-47.5	50 ACN: 50 0.1% PCA	Agilent Zorbax Bonus RP (250 x 4.6mm, 5µ)	250	2.89	12778	1.03	0.00	4.37	6152	0.87	9.06
4	ACN : 0.1% PCA	52.5-47.5	50 ACN: 50 0.1% PCA	Phenomenex Kinetex XB-C18 (150 x 4.6mm, 5µ)	250	1.55	5845	1.04	0.00	4.19	6565	0.93	18.38

The chromatograms of different trials are depicted in figure 2 to figure 5.

Figure 2: Method development trial 1

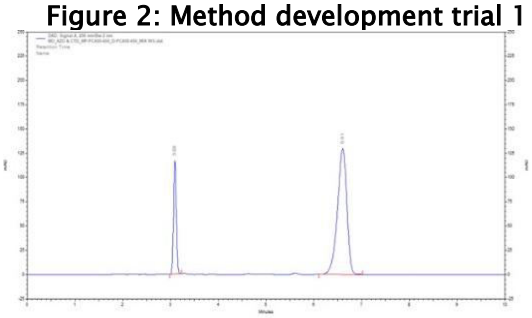


Figure 3: Method development trial 2

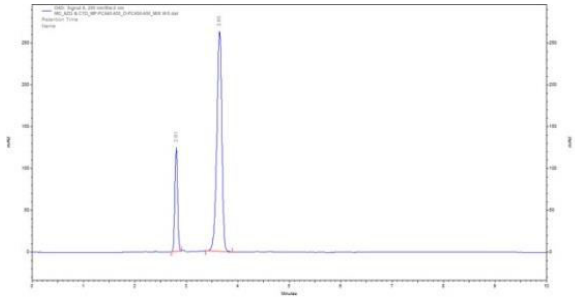


Figure 4: Method development trial 3

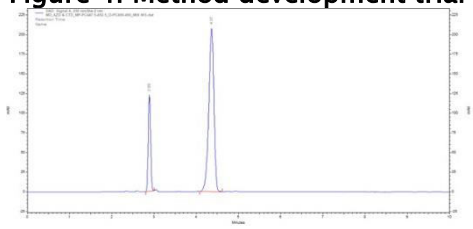
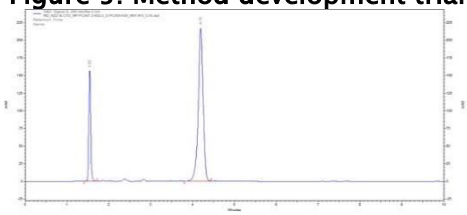


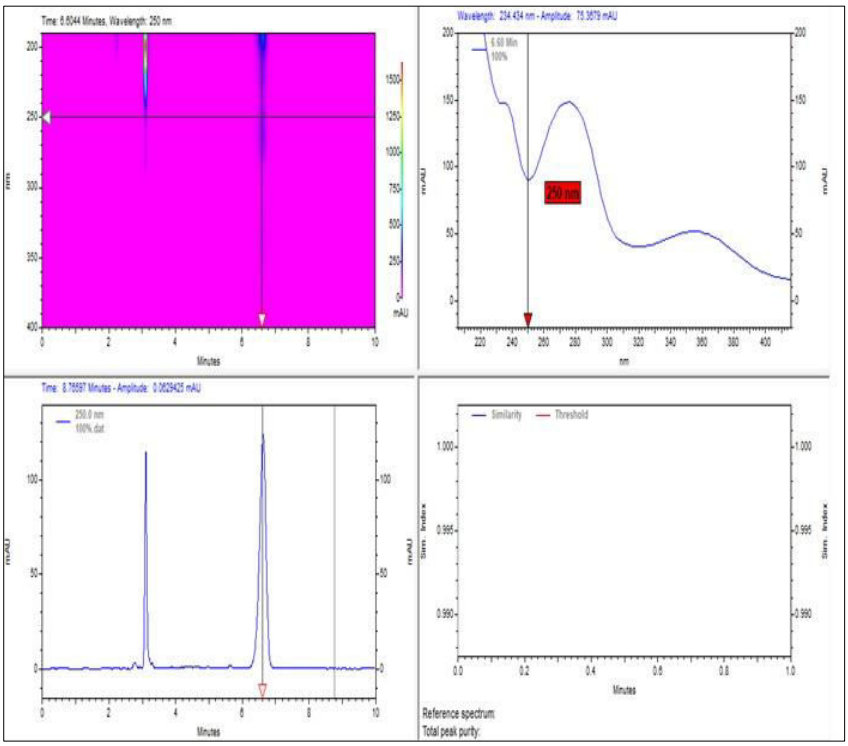
Figure 5: Method development trial 4



II. Selection of wavelength

The Wavelength for the determination of Chlorthalidone and Azelnidipine was selected at 270 nm on the basis of appropriate intensity of both the peaks. Mixed DAD Spectrum for Wavelength Selection is shown in figure 6.

Figure 6 – Mixed DAD Spectrum for Wavelength Selection



III. Method validation[25-28]

Specificity

Individual working standards of Chlorthalidone and Azelnidipine as well as Mixture Working standard were run on HPLC and for the identification of peaks on the basis of retention time. No Significant peaks of were seen in the chromatogram due to the blank. The retention time of Chlorthalidone peak was found to be 3.11 minutes. The retention time of Azelnidipine peak was found to be 6.68 minutes. It can be seen that there is no interaction between both the API peaks. Also, there is no interference of diluent with any of the APIs peaks. The chromatograms for specificity studies are shown in figure 7 to figure 10.

Figure 7: Chromatogram of diluent

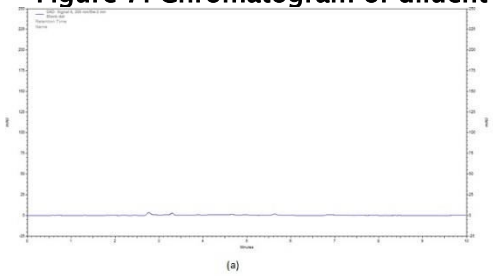


Figure 8: Chromatogram of standard Chlorthalidone

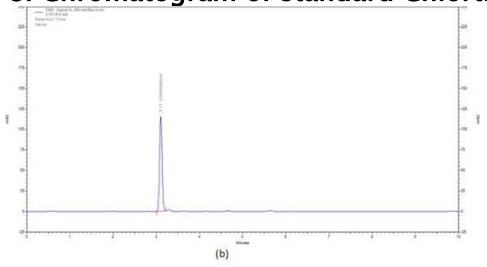


Figure 9: Chromatogram of standard Azelnidipine

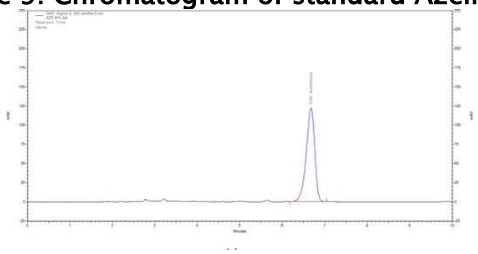
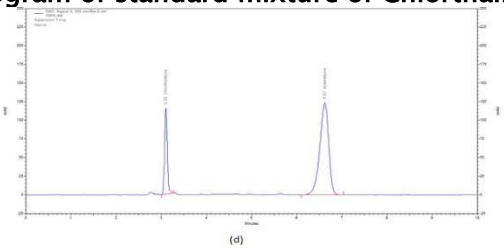


Figure 10: Chromatogram of standard mixture of Chlorthalidone and Azelnidipine



Linearity

The 5 points Linearity was performed for each Working Standard API. The results obtained are detailed below.

Linearity for Chlorthalidone

Table 2: Linearity –Chlorthalodone

% Level	Conc (µg/ml)	Area
80	50	807874
90	56.25	904100
100	62.5	993603
110	68.75	1117061
120	75	1221475

Figure 11 : Linearity Chlorthalidone

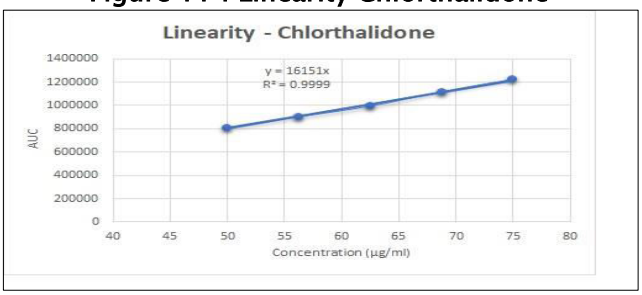


Table 3: Linearity – Azelnidipine

% Level	Conc (µg/ml)	Area
80	64	2832435
90	72	3188408
100	80	3525683
110	88	3772935
120	96	4316534

Figure 12 : Linearity Azelnidipine

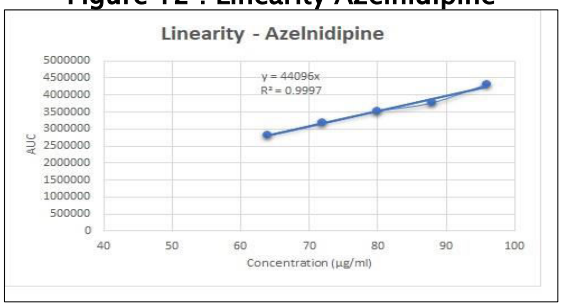
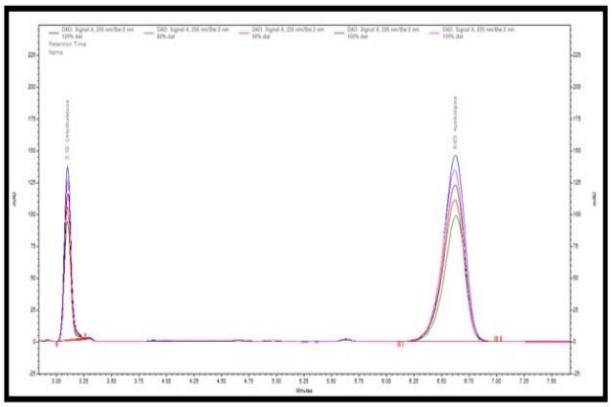


Figure 13: Linearity Overlay



Range

The range of linearity and analysis for Chlorthalidone and Azelnidipine was found to be 50-75 µg/ml and 64-96 µg/ml respectively.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ values were obtained are given below.

Table 4: LOD and LOQ values

Name of API	Chlorthalidone	Azelnidipine
LOD (µg/ml)	1.1	1.13
LOQ (µg/ml)	3.33	3.41

Accuracy

Accuracy study was performed for at 80%, 100% and 120% level. The data is reported in table 5 and table 6.

Table 5: Accuracy study data for Chlorthalidone

Conc Levels	Reps	Spiked Conc.	Area	Amount Recovered	% Recovery	AVG	STD Deviation	% RSD
80%	Rep 1	50	807874	49.82	99.64	99.62	0.020311	0.02
	Rep 2	50	807545	49.8	99.6			
	Rep 3	50	807696	49.81	99.61			
100%	Rep 1	62.5	1013603	62.5	100.01	100	0.021022	0.02

	Rep 2	62.5	1013251	62.48	99.97			
	Rep 3	62.5	1013635	62.51	100.01			
120%	Rep 1	75	1221475	75.32	100.43	100.46	0.048349	0.05
	Rep 2	75	1222493	75.39	100.51			
	Rep 3	75	1221474	75.32	100.43			

Table 6: Accuracy study data for Azelnidipine

Conc Levels	Reps	Spiked Conc.	Area	Amount Recovered	% Recovery	AVG	STD Deviation	% RSD
80%	Rep 1	6.4	2832435	6.4	100.06	100.62	0.485161	0.48
	Rep 2	6.4	2857441	6.46	100.94			
	Rep 3	6.4	2854779	6.45	100.85			
100%	Rep 1	8	3525683	7.97	99.64	99.62	0.016899	0.02
	Rep 2	8	3524751	7.97	99.62			
	Rep 3	8	3524568	7.97	99.61			
120%	Rep 1	9.6	4216534	9.53	99.31	99.25	0.062707	0.06
	Rep 2	9.6	4211223	9.52	99.18			
	Rep 3	9.6	4214214	9.53	99.25			

All the %RSD for 80%, 100% and 120% are within the specification of less than 2%. The method is accurate for Chlorthalidone and Azelnidipine.

Precision (Repeatability)

A total of 6 replicate injections were done to check if the method is precise or not. The data was analysed for deviations. The precision study data is given in table 7.

Table 7: Repeatability study data

Name	Chlorthiazide	Azelnidipine
Reps	**Area**	**Area**
Rep 1	1013603	3525683
Rep 2	1013251	3524751
Rep 3	1013635	3524568
Rep 4	1013254	3521476
Rep 5	1013057	3533865
Rep 6	1014354	3599872
Avg	1013526	3538369
STDEV	463.2415	30414.24
RSD	0.05	0.86

Assay

Drug product tablet was injected and compared with the Working standard chromatogram. Assay was calculated based on area of each identified drug peak.The results for assay are given in table 8. The % Assay for Chlorthalidone and Azelnidipine was found to be 98.93% and 98.90% respectively.

Table 8: Data for assay of Chlorthalidone and Azelnidipine by developed method

Sample ID	Chlorthalidone (CTD)	Azelnidipine (AZD)

	RT	Area	% Assay	RT	Area	% Assay
CTD WS	3.11	976786	–	–	–	–
AZD WS	–	–	–	6.68	3549333	–
MIX WS	3.10	1013526	–	6.62	3538369	–
Drug Product	3.10	1002647	98.93	6.62	3499335	98.90

System Suitability

System suitability was performed as per instruction in Material and Method and results were recorded as below in table 9 and 10.

Sample ID	RT	Theoretical Plate	Asymmetry	Resolution
100% Rep 1	3.10	12747	1.16	0.00
100% Rep 2	3.10	12804	1.12	0.00
100% Rep 3	3.10	12902	1.20	0.00
100% Rep 4	3.10	12750	1.11	0.00
100% Rep 5	3.10	12969	1.21	0.00
100% Rep 6	3.10	12754	1.10	0.00
AVG	3.10			
STD DEV	0			
% RSD	0.00			

Table 9: System Suitability data for Chlorthalidone

Table 10: System suitability data for Azelnidipine

Sample ID	RT	Theoretical Plate	Asymmetry	Resolution
100% Rep 1	6.62	5384	0.86	14.96
100% Rep 2	6.62	5382	0.82	14.96
100% Rep 3	6.62	5380	0.86	14.96
100% Rep 4	6.62	5386	0.92	14.96
100% Rep 5	6.62	5374	0.83	14.96
100% Rep 6	6.62	5396	0.89	14.96
AVG	6.62			
STD DEV	0			
% RSD	0.00			

It can be inferred that the % Relative Standard Deviation for Retention time of each drug was found to be 0.00 which means that there is no change in Retention time of any of the drug for 6 repetitions. The Asymmetry of both the drugs were within the ICH guidelines which is less than 2. The theoretical plates for each API for all reps are above 2000 as per ICH Guidelines. Resolution is above 2 for Azelnidipine which is as per the specifications.

Conclusion

This research was aimed to develop and validate a method for the estimation of Azelnidipine and Chlorthalidone in bulk and tablet Formulation. The proposed method was found to be appropriate

due to its simplicity, reliability, sensitivity, rapidness and selectivity for detection at very low concentrations. The short chromatographic time makes this method suitable for processing of multiple samples in short time. The method showed no interference of the excipients present in Drug Product of Azelnidipine and Chlorthalidone. The statistical parameters and recovery data reveals the good accuracy and precision of the proposed method. The RP-HPLC method developed for the estimation of Azelnidipine and Chlorthalidone was validated as per the ICH guidelines.
Validation data demonstrates that, this method is accurate, precise, simple, economic as well as robust and can be used in the routine analysis of Azelnidipine and Chlorthalidone in various formulations.

Acknowledgments: None.
Conflict of interest: None.
Financial support: None**Ethics statement**: None.

References

18

1. Shewale VU, Aher SS, Saudagar RB. Azelnidipine: a review on therapeutic role in hypertension. Journal of Drug Delivery and Therapeutics. 2019 May 2;9(3s):1002-5.
2. Xiao Y, Hu G. The effects of azelnidipine and amlodipine in treatment of mild to moderate hypertension: a systematic review. Int J Clin Exp Med. 2017 Jan 1;10(7):11273-81.
3. Aher SS, Saudagar RB. Azelnidipine: A review on therapeutic role in hypertinsion. Journal of Drug Delivery and Therapeutics. 2019 Jun 15;9(3-s):1002-5.
4. Neff KM, Nawarskas JJ. Hydrochlorothiazide versus chlorthalidone in the management of hypertension. Cardiology in Review. 2010 Jan 1;18(1):51-6.

5. Hripcsak G, Suchard MA, Shea S, Chen R, You SC, Pratt N, Madigan D, Krumholz HM, Ryan PB, Schuemie MJ. Comparison of cardiovascular and safety outcomes of chlorthalidone vs hydrochlorothiazide to treat hypertension. JAMA internal medicine. 2020 Apr 1;180(4):542-51.
6. Kostis JB, Cabrera J, Cheng JQ, Cosgrove NM, Deng Y, Pressel SL, Davis BR. Association between chlorthalidone treatment of systolic hypertension and long-term survival. Jama. 2011 Dec 21;306(23):2588-93.
7. Okamura K, Yano Y, Takamiya Y, Shirai K, Urata H. Efficacy and safety of a combination antihypertensive drug (olmesartan plus azelnidipine):"issues with hypertension studies in real-world practice". Clinical and Experimental Hypertension. 2020 Jul 3;42(5):438-48.
8. Riva N, YH Lip G. Which is the optimal antihypertensive combination in different diseases, a renin-angiotensin-aldosterone system inhibitor with a diuretic or with a calcium channel blocker?. Current pharmaceutical design. 2013 Jun 1;19(21):3753-65.
9. Nagasu H, Satoh M, Yorimitsu D, Tomita N, Sasaki T, Kashihara N. Comparison of combination therapy of olmesartan plus azelnidipine or hydrochlorothiazide on renal and vascular damage in SHR/NDmcr-cp rats. Kidney and Blood Pressure Research. 2011 Jan 27;34(2):87-96.
10. Chen BL, Zhang YZ, Luo JQ, Zhang W. Clinical use of azelnidipine in the treatment of hypertension in Chinese patients. Therapeutics and clinical risk management. 2015 Feb 24:309-18.
11. Fujiwara T, Kario K. Outcomes of Different Antihypertensive Regimens. Management of Hypertension: Current Practice and the Application of Landmark Trials. 2019:183-214.
12. Narita K, Hoshide S, Kario K. Polypill Therapy for Cardiovascular Disease Prevention and Combination Medication Therapy for Hypertension Management. Journal of Clinical Medicine. 2023 Nov 22;12(23):7226.
13. Kanno Y, Ohno Y, Takenaka T. Calcium Channel Blockers in the Treatment of Hypertension. Pathophysiology and Pharmacotherapy of Cardiovascular Disease. 2015:807-22.
14. Redon J, Carmena R. Present and future of drug therapy in hypertension: an overview. Blood Pressure. 2024 Dec 31;33(1):2320401.
15. Matsui Y, Eguchi K, O'Rourke MF, Ishikawa J, Miyashita H, Shimada K, Kario K. Differential effects between a calcium channel blocker and a diuretic when used in combination with angiotensin II receptor blocker on central aortic pressure in hypertensive patients. Hypertension. 2009 Oct 1;54(4):716-23.
16. PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 65948, Azelnidipine; [cited 2024 May 8]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Azelnidipine
17. PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 2732, Chlorthalidone; [cited 2024 May 8]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Chlorthalidone
18. Raimalani J, Kotadiya R. Progress in quantitative methods for azelnidipine and chlorthalidone: an analytical basis for a recently approved FDC. Current Pharmaceutical Analysis. 2023 Jan 1;19(1):66-82.
19. Sharma S. Newer drug choices in hypertension treatment. Hypertension. 2020 Apr;6(2):70-3.

20. Kanu AB. Recent developments in sample preparation techniques combined with high-performance liquid chromatography: A critical review. Journal of Chromatography A. 2021 Sep 27;1654:462444.
21. Thakker NM, Choudhari VP, Kuchekar BS, Panchal HB, Rakholiya DR, Murugan R. Development and validation of a stability indicating RP-HPLC method for simultaneous estimation of Olmesartan Medoxomil and Metoprolol Succinate in pharmaceutical dosage form. Pharmaceutical Methods. 2012 Jul 1;3(2):84-9.
22. Sahana KM, GS NK, Suresh DN. Definitive Review of Analytical Methods Reported on Estimation of Azelnidipine. Journal of Pharma Insights and Research. 2024 Feb 6;2(1):108-12.
23. Ravisankar P, Navya CN, Pravallika D, Sri DN. A review on step-by-step analytical method validation. IOSR J Pharm. 2015 Oct;5(10):7-19.
24. Kumar SD, Kumar DH. Importance of RP-HPLC in analytical method development: a review. International journal of pharmaceutical sciences and research. 2012 Dec 1;3(12):4626.
25. Guideline IH. Validation of analytical procedures: text and methodology. Q2 (R1). 2005 Nov;1(20):05.
26. Kalra K. Method development and validation of analytical procedures. Quality Control of Herbal Medicines and Related Areas. 2011 Nov 4;4:3-16.