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"Development and Validation of an Analytical Method for Simultaneous EstimationofAzelnidipine and Chlorthalidone by UV in Fixed -Dose Combination"

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ABSTRACT:-
A UV spectrophotometric approach for quantifying Azelnidipine and Chlorthalidone in fixed-dose combinations that is simple, fast, sensitive, accurate, and exact. The absorbance of Azelnidipine and Chlorthalidone was measured at two different wavelengths 260 nm and 227 nm, respectively. The isosbestic point was discovered to be 250 nm in diameter. Azelnidipine (r2=0.9994) and Chlorthalidone (r2=0.9991) both showed linearity in the 64µg/ml to 96µg/ml and 50µg/ml to 75µg/ml range respectively. A recovery study was carried out to confirm the methods' accuracy. In the recovery study, the % RSD was less than 2. The methods were validated in accordance with ICH guidelines.

KEYWORDS:-Azelnipidine, Chlorthalidone, Simultaneous Estimation, UV

I. INTRODUCTION:-

Cardiovascular disease (CVD) is the leading cause of death in the elderly, and it is on the rise in developing countries. Blood pressure control is an important part of CVD management. According to WHO, more than 75% of CVD events could be avoided. [1] A fixed-dose combination of calcium channel blockers (Azelnidipine) and diuretics (Chlorthalidone) was found to reduce the risk of stroke, myocardial infraction, and cardiovascular mortality. The use of a combination of different antihypertensive agents with different mechanisms of action reduced CVD mortality and morbidity. [2]

Chemically, Azelnidipine is (±)-3-(1-diphenylmethylazetidin-3-yl) 5-isopropy12-amino-1, 4-dihydro-6-methyl-4-amino-1, 4-dihydro-6-methyl-4-amino-1, 4-dihydro-6-methyl-4-amino-1, 4-dihydro-6-methyl-4-amino-1, 4-di (3-nitrophenyl) pyridinedicarboxylate (-3,5-pyridinedicarboxylate) (fig 1). It is a calcium channel blocker (CCB) of the dihydropyridine (DHP) type used to treat hypertension. [3] Due to an asymmetric carbon at the 4-position of the DHP ring, AZEL contains two enantiomers. The (R)-enantiomer of Azelnidipine has pharmacological activity. [4] This is in contrast to other CCBs, where the biological action is attributed to the (S)-enantiomer. The unusual three-dimensional structure of Azelnidipine's active enantiomer may be linked to its unique pharmacological properties, such as long-lasting blood pressure reduction, lowered heart rate, and antiatherosclerotic action, which are not shared by other DHPs. Azelnidipine also has a diuretic effect, increasing urine volume and thereby lowering blood pressure. [5, 6]

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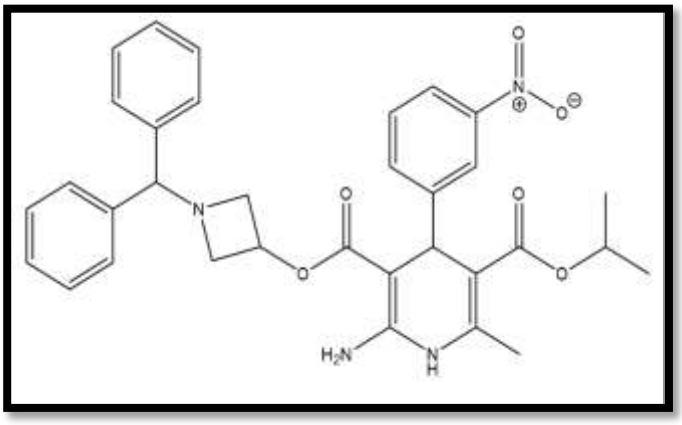


FigureNo. 1:- Structure of Azelnidipine

Chemically, Chlorthalidoneis (RS)-2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoondol-1-yl) benzene-1-sulfonamide (Fig 2), is a drug that is commonly used to treat hypertension and edema. [7]It's a diuretic that prevents sodium from being transported across the renal tubular epithelium in the ascending limb of the loop of the Henle's cortical diluting segment. Chlorthalidone has a longer duration of action than other medications in the thiazide class, but it has a similar diuretic effect at therapeutic levels. [8]

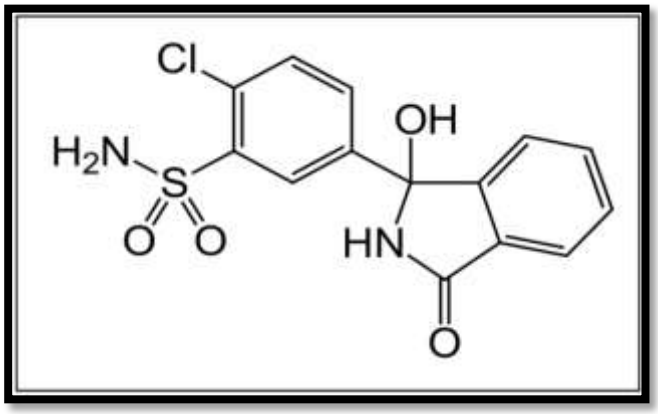


Figure No.2:-StructureofChlorthalidone

A review of the literature revealed that some methods for simultaneous estimation [9] of these drugs as well as methods for individual drug estimation have been reported. UV-Spectrophotometry, RP-HPLC, or other drugs. In the literature, there is no spectrophotometric method for UV simultaneous estimation of Azelnidipine and Chlorthalidone. As a result, we decided to create and validate a new simple, quick, accurate, specific, highly sensitive, and simultaneous determination for Azelnidipine and Chlorthalidone estimation. The method was validated in accordance with ICH guidelines.

II. MATERIALANDMETHOD:

Instrument:

AShimadzu1800UV/VISdoublebeamspectrophotometer with 1 cm matched quartz cellswasusedfor different derivative spectral measurements. The UV spectra were recorded over the wavelength of 200-400nm. All the drugs and chemicals were weighed the on metter torledo model weighing balance.

ChemicalsandReagents:

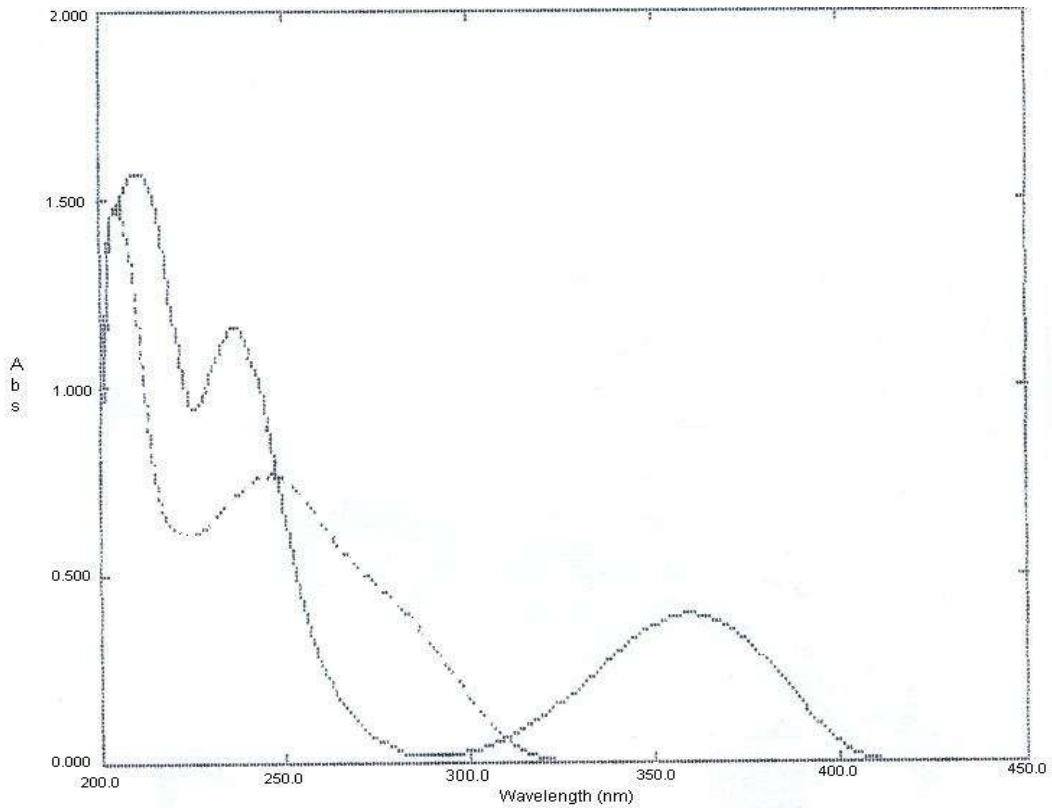
A gift sample of analytically pure Azelnidipine and Chlorthalidone was received from Aadhar Life Science Pvt Ltd. M. I. D. C, Solapur was used in

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 1265-1275 www.ijprajournal.com ISSN: 2456-4494

the study. The solvent used was Methanol and Distilled water was used in the preparationof the mobilephase.

Selectionof Wavelength:

The sample was scanned from 200-400 nm with PDA detector. The Wavelength selected for analysis chosen was 250 nm on basis of appropriate intensity of both the peaks.



FigureNo.3:-Isosbesticpoint ofAzelnidipine and Chlorthalidone

PreparationofstocksolutionofAzelnidipine:

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

Preparation of stock solution of Chlorthalidone:

Initially Prepare a Standard Stock Solution (SSS-II) of Chlorthalidone by adding 6.25 mg in a 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Chlorthalidone = 625 µg/ml).

ASSAY OF TABLET:

A Tablet sample of fixed dose combination was prepared for assay. Due to unavailability of the dosage form individual tablets of Azelnidipine and Chlorthalidone were used to simulate the conditions of actual product. Tablet powder equivalent to8 mg of Azelnidipine and 6.25 mg Chlorthalidone and was weighed and mixed with diluent and sonicate for 5 minutes. (Stock conc of Azelnidipine = 800 µg/ml and Chlorthalidone = 625 µg/ml). 1 ml of above solution was further diluted to 10 ml (Conc of & Azelnidipine = 80 µg/ml and Chlorthalidone = 62.5 µg/ml). Individual samples of and Azelnidipine and Chlorthalidone were prepared of 80 µg/ml and 62.5 µg/ml, respectively**.**

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 1265-1275 www.ijprajournal.com ISSN: 2456-4494

Sample ID	Azelnidipine			Chlorthalidone		
	Absorbance	Amount Recovered (µg/ml)	% Recovery	Absorbance	Amount Recovered (µg/ml)	% Recovery
WS	0.635	-	-	0.565	-	-
DP-1	0.625	78.74	98.43	0.563	62.32	99.70
DP-2	0.617	77.73	97.17	0.531	62.09	99.35
DP-3	0.632	79.62	99.53	0.566	62.65	100.24
DP-4	0.627	78.99	98.74	0.561	62.09	99.35
DP-5	0.629	79.24	99.06	0.569	62.98	100.77
AVG	0.626	78.866	98.583	0.564	62.426	99.882
STDEV	0.005656854	0.712674551	0.890843	0.003464102	0.38342329	0.613477
% RSD	0.90	0.90	0.90	0.61	0.61	0.61

Table.1: AssayofAzelnidipine and Chlorthalidone

SimultaneousEstimationof Azelnidipine and Chlorthalidone:

In the simultaneousmethod,weusedabsorbance at two selected wavelengths. To determine the λmaxof boththe drugs we scan in the range of 200-400nm.Standardsolutionsofdifferentconcentrations ofbothdrugswerepreparedin the mobilephase.The absorbanceofAzelnidipine(800µg/ml) and Chlorthalidone (625µg/ml) wererecorded at two wavelengths 260nm and 227 nm by using the simultaneous equationmethod.

Cx = A2ay1- A1ay2/ ax2ay1- ax1ay2 and

CY = A1ax2- A2ax1/ ax2ay1- ax1ay2

Where,

Cx= concentration of Azelnidipine

Cy= concentration of Chlorthalidone

ax1and ax2= absorptivity value of Azelnidipine at 260nm and 227 nm

ay1and ay2= absorptivity value of Chlorthalidone at 260 nm and 227 nm

A1=absorbance of the standard mixture at 260nm

A2=absorbance of the standard mixture at 227nm

III. METHODVALIDATION:-

Validation is the process of establishing documented evidence that provide a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes. The present method was validated as per ICH guidelines.The parameter evaluated were linearity,accuracy,precision,LOD,andLOQ.

Linearity:

Linearity was studied by plotting a graphofabsorbancedirectlyproportionaltotheconcentration.Aseriesofstandardsolutionsof Azelnidipine concentration range is 64µg/ml to 96 µg/ml and Chlorthalidone waspreparedintheconcentration range of about 50 µg/ml to 75 µg/ml isshowninbelowtables(2) & (3). The absorbance values for Azelnipidine and chlorthalidone were measured at respective wavelength for each drug separately.

Azelnidipine		
% Level	Conc (µg/ml)	Abs
80	64	0.513
90	72	0.568
100	80	0.633

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 1265-1275 www.ijprajournal.com ISSN: 2456-4494

110	88	0.696
120	96	0.757

Table No. 2:- Linearity study of Azelnidipine

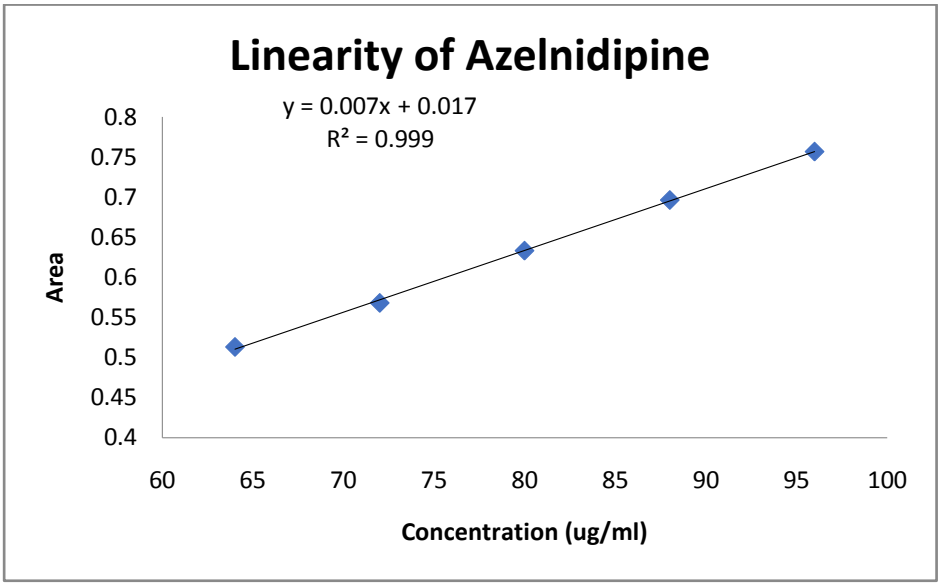


Figure No. 6:- Linearity Graph of Azelnidipine

Chlorthalidone		
% Level	Conc (µg/ml)	Abs
80	50	0.453
90	56.25	0.504
100	62.5	0.568
110	68.75	0.625
120	75	0.682

Table No. 3:- Linearity study of Chlorthalidone

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 1265-1275 www.ijprajournal.com ISSN: 2456-4494

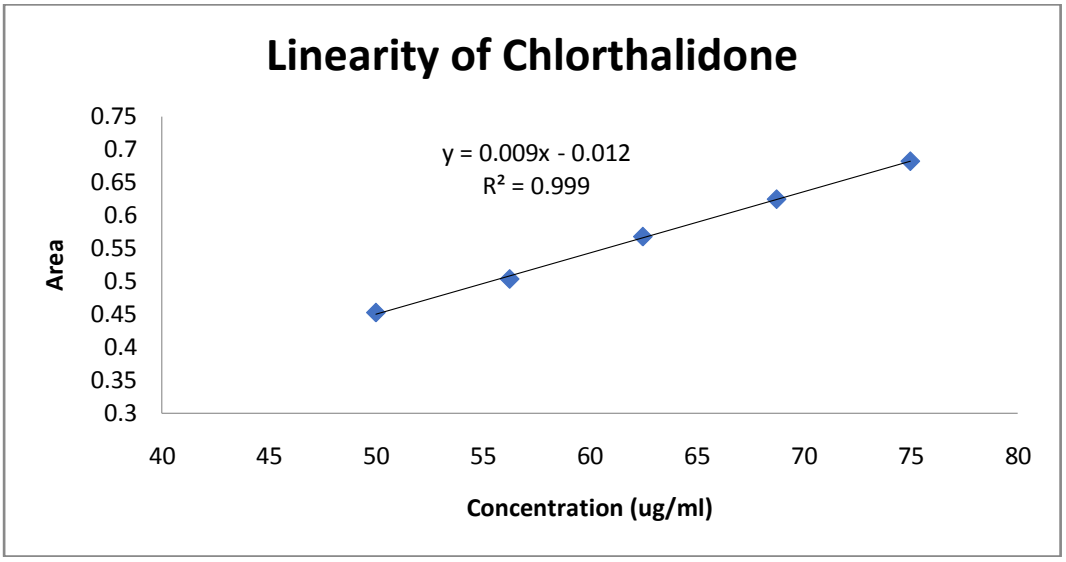


Figure No. 7:- Linearity Graph of Chlorthalidone

Precision:

Sixseparatesolutionscomprisingconcentrations of 72, 80, and 88 µg/ml of Azelnidipine and 56.25,62.5, and 68.75 µg/ml of Chlorthalidonewereanalyzedforrepeatability.Theabsorbance was measured three times each day to determineintra-day and inter-day variation. The %RSD wasdetermined to be less than 2, shown in the below tables (4,5,6, 7).

Azelnidipine						
Conc (µg/ml)	**Absorbance**			**Avg**	**STDEV**	**RSD**
	Trial 1	**Trial 2**	**Trial 3**			
72	0.569	0.567	0.561	0.565667	0.00	0.74
80	0.631	0.637	0.635	0.634333	0.00	0.48
88	0.694	0.692	0.689	0.691667	0.00	0.36

Table No. 4:- Intra-day precision of Azelnidipine

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 1265-1275 www.ijprajournal.com ISSN: 2456-4494

Azelnidipine						
Conc (µg/ml)	Absorbance			Avg	STDEV	RSD
	Trial 1	Trial 2	Trial 3			
72	0.568	0.569	0.566	0.568	0.0015	0.27
80	0.633	0.631	0.629	0.631	0.0020	0.32
88	0.696	0.697	0.692	0.695	0.0026	0.38

Table No. 5:- Inter-day precision of Azelnidipine

Chlorthalidone						
Conc (µg/ml)	Absorbance			Avg	STDEV	RSD
	Trial 1	Trial 2	Trial 3			
56.25	0.503	0.503	0.509	0.505	0.00	0.69
62.5	0.571	0.573	0.576	0.573333	0.00	0.44
68.75	0.624	0.622	0.629	0.625	0.00	0.58

Table No.6:- Intra-day precision of Chlorthalidone

Chlorthalidone						
Conc (µg/ml)	Absorbance			Avg	STDEV	RSD
	Trial 1	Trial 2	Trial 3			
56.25	0.504	0.501	0.506	0.504	0.0025	0.50
62.5	0.568	0.569	0.566	0.568	0.0015	0.27
68.75	0.625	0.623	0.621	0.623	0.0020	0.32

Table No.7:- Intra-day precision of Chlorthalidone

Accuracy:

This parameter is performed to determinethe closeness of the test results with that of the truevaluewhichisexpressedas%recovery. Recovery studies were carried outatthreedifferentlevels(80%,100%,and120%) by spiking the same amount of concentration given above in the table for both Azelnidipine and Chlorthalidone. Samples were analysed in Triplicate to calculate % RSD. The % recovery was also calculated below table no. (8 and 9)

Azelnidipine			
Std wt. (mg)	% Purity	Std Stock Conc. (µg/ml)	Working Std Area
8	100	80	0.635

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 1265-1275 www.ijprajournal.com ISSN: 2456-4494

Sample ID	Reps	Spiked Conc. (µg/ml)	Area	Amount Recovered (µg/ml)	% Recovery	AVG	STDEV	% RSD
80%	Rep 1	6.40	0.513	6.46	100.98	100.66	0.409777	0.41
	Rep 2	6.40	0.512	6.45	100.79			
	Rep 3	6.40	0.509	6.41	100.20			
100%	Rep 1	8.00	0.633	7.97	99.69	99.74	0.240555	0.24
	Rep 2	8.00	0.632	7.96	99.53			
	Rep 3	8.00	0.635	8.00	100.00			
120%	Rep 1	9.60	0.757	9.54	99.34	99.65	0.330264	0.33
	Rep 2	9.60	0.759	9.56	99.61			
	Rep 3	9.60	0.762	9.60	100.00			

Table No. 8:- Recovery Study of Azelnidipine

Chlorthalidone			
Std wt. (mg)	**% Purity**	**Std Stock Conc. (µg/ml)**	**Working Std Area**
6.25	100	62.5	0.565

Sample ID	Reps	Spiked Conc. (µg/ml)	Area	Amount Recovered (µg/ml)	% Recovery	AVG	STDEV	% RSD
80%	Rep 1	5.00	0.453	5.01	100.28	100.35	0.338148	0.34
	Rep 2	5.00	0.452	5.00	100.06			
	Rep 3	5.00	0.455	5.04	100.72			
100%	Rep 1	6.25	0.568	6.29	100.59	99.82	0.670473	0.67
	Rep 2	6.25	0.562	6.22	99.53			
	Rep 3	6.25	0.561	6.21	99.35			
120%	Rep 1	7.50	0.682	7.55	100.65	101.53	0.821689	0.81
	Rep 2	7.50	0.689	7.63	101.68			
	Rep 3	7.50	0.693	7.67	102.27			

Table No. 9:- Recovery Study of Chlorthalidone

Robustness:

The robustness of an analytical technique is a measure of its ability to remain unaffected by small but deliberate changes in method parameters, and it provides an indication of its dependability in routine use. The method's robustness was investigated for Azelnidipine and Chlorthalidone.

LOD/ LOQ:

The limit of Quantitation is 3 times more than the

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limitof detection respectively. The LOD value of Azelnidipine and Chlorthalidone was found to be 3.84 µg/ml and 3.54µg/ml respectively and the LOQ value of Azelnidipine and Chlorthalidone were foundtobe11.63µg/mland10.74µg/ml respectively.

It was calculated for both drugs by using the ANOVA technique.

Formula:

$$\mathbf{LOD} = \frac{3.3 \times \text{Std. Error of Intercept}}{\text{Coefficients of X Variable 1}}$$

$$\mathbf{LOQ} = \frac{10 \times \text{Std. Error of Intercept}}{\text{Coefficients of X Variable 1}}$$

SrNo.	Nameofdrugs	LOD (µg/ml)	LOQ(µg/ml)
1	Azelnidipine	3.84	11.63
2	Chlorthalidone	3.54	10.74

Table No.10:- Result of LOD and LOQ

IV. RESULT AND DISCUSSION:

Theproposedmethodisbasedonspectrophotometricsimultaneousestimationof Azelnidipine and Chlorthalidone inthismethodmethanolanddistilledwaterisusedassolvent.

Linearity

Linear regression data for the calibration plots revealed good linear relationship between absorbance and concentration over the ranges 64µg/ml to 96 µg/ml of Azelnidipine and 50µg/ml to 75µg/ml of Chlorthalidone. The linear equation for the calibration plots were y = 0.0077x + 0.00174andy = 0.0093x - 0.00126with Regression(R^2) being 0.9994 and 0.9991for Azelnidipine and Chlorthalidone, respectively. (Figure1 and 2) (Table 2 and 3)

Precision

The precision of method was expressed as relative standard deviation (RSD%). The %RSD values for intra-day precision study and intra-day study listed in (Table 4,5,6 and 7)were < 2 %, confirming that the method was sufficiently precise.

Accuracy

When the method was used for accuracy and subsequent analysis of both the drugs from the pharmaceutical dosage form and spiked with 80, 100, 120% of additional pure drug, the recovery was found to be100.66% and 99.65% for Azelnipidine and 100.35% and 101.53% for Chlorthalidone.(Table No. 8 and 9)

LOD and LOQ

The LOD and LOQ were calculated by equation. The LOD and LOQ values were 3.84µg/ml and 11.63 µg/ml for Azelnidipine and 10.74 µg/ml and 3.54 µg/ml for chlorthalidone.(Table No.10)

V. CONCLUSION

The proposed method was developed for the determination of Azelnidipine and Chlorthalidone in the presence of each other. Methods was validated and found to be simple, rapid, sensitive, accurate and precise. The short chromatographic time makes this method suitable for processing of multiple samples in short time. The method shows no interference by the excipients. This method can be useful and suitable for the estimation of Azelnidipine and Chlorthalidone in bulk and pharmaceutical dosage form.

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