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DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE AND CHLORTHALIDONE BY UV IN FIXED DOSE COMBINATION

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Abstract: A UV spectrophotometric approach for quantifying Amlodipine and Chlorthalidone in fixed-dose combinations that is simple, fast, sensitive, accurate, and exact. The absorbance of Amlodipine and Chlorthalidone was measured at 260 nm and 227nm respectively. The isosbestic point was discovered to be 250 nm in diameter. Amlodipine (r2=0.9994) and Chlorthalidone (r2=0.999) both showed linearity in the 40µg/ml to 60µg/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: Amlodipine, Chlorthalidone, Simultaneous Estimation, UV Spectrophotometric Method

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 (Amlodipine) 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, Amlodipine is (RS)-3-ethyl 5-methyl(±)2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate monobenzenesuphonate. (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 Amlodipine 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 Amlodipine'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. Amlodipine also has a diuretic effect, increasing urine volume and thereby lowering blood pressure. [5, 6]

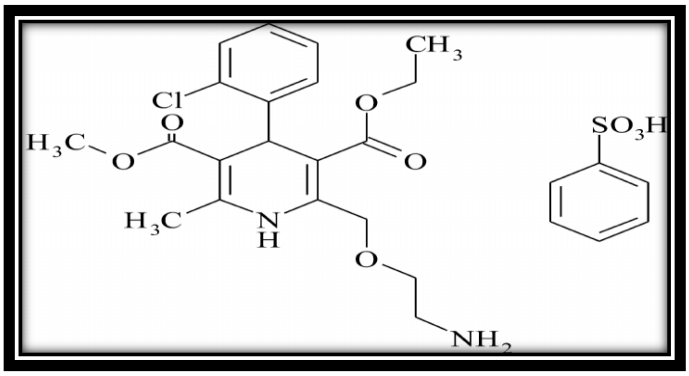

Figure No. 1: - Structure of Amlodipine

Chemically, Chlorthalidone is (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 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]

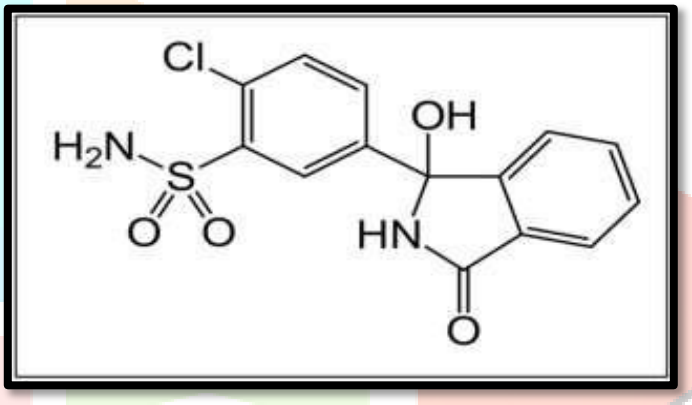

Figure No.2: -Structure of Chlorthalidone

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 Amlodipine and Chlorthalidone. As a result, we decided to create and validate a new simple, quick, accurate, specific, highly sensitive, and simultaneous determination for Amlodipine and Chlorthalidone estimation. The method was validated in accordance with ICH guidelines.

II. MATERIAL AND METHOD:

Instrument:

A Shimadzu 1900UV/VIS double beam spectrophotometer with 1 cm matched quartz cells was used for different derivative spectral measurements. The UV spectra were recorded over the wavelength of 200-400nm. All the drugs and chemicals were weighed the on meter torledo model weighing balance.

Chemicals and Reagents:

A gift sample of analytically pure Amlodipine and Chlorthalidone was received from Zydus life sciences Pvt. Ltd. Ahmadabad, Gujrat was used in the study. The solvent used was Methanol and Distilled water was used in the preparation of the mobile phase.

Selection of 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.

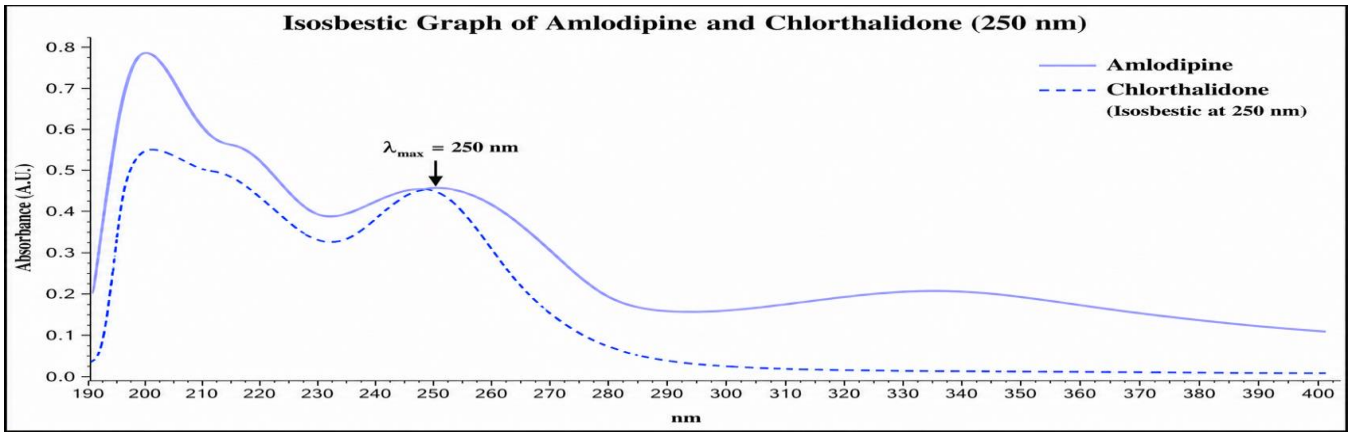

Figure No.3: - Isosbestic point of Amlodipine and Chlorthalidone

Preparation of stock solution of Amlodipine:

Prepare a Standard Stock Solution (SSS-I) of Amlodipine by adding 5 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 Amlodipine = 500 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).

III. ASSAY OF TABLET

A Tablet sample of fixed dose combination was prepared for assay. Due to unavailability of the dosage form individual tablets of Amlodipine and Chlorthalidone were used to simulate the conditions of actual product. Tablet powder equivalent to 5 mg of Amlodipine and 6.25 mg Chlorthalidone and was weighed and mixed with diluent and sonicate for 5 minutes. (Stock conc of Amlodipine = 500 µg/ml and Chlorthalidone = 625 µg/ml). 1 ml of above solution was further diluted to 10 ml (Conc of & Amlodipine = 50 µg/ml and Chlorthalidone = 62.5 µg/ml). Individual samples of and Amlodipine and Chlorthalidone were prepared of 50 µg/ml and 62.5 µg/ml, respectively.

Table.1: Assay of Amlodipine and Chlorthalidone

Sample ID	Amlodipine			Chlorthalidone		
	Absorbance	Amount Recovered (µg/ml)	% Recovery	Absorbance	Amount Recovered (µg/ml)	% Recovery
WS	0.397	-	-	0.565	-	-
DP-1	0.401	50.50	101.01	0.563	62.32	99.70
DP-2	0.403	50.76	101.51	0.531	62.09	99.35
DP-3	0.406	51.13	102.27	0.566	62.65	100.24
DP-4	0.406	51.13	102.27	0.561	62.09	99.35
DP-5	0.406	51.13	102.27	0.569	62.98	100.77
AVG	0.404	50.932	101.864	0.564	62.426	99.882
STDEV	0.002302173	0.289946207	0.579892	0.003464102	0.38342329	0.613477
% RSD	0.57	0.57	0.57	0.61	0.61	0.61

Simultaneous Estimation of Amlodipine and Chlorthalidone:

In the Simultaneous method, we used absorbance at two selected wavelengths. To determine the λmax of both the drugs we scan in the range of 200-400nm. Standard solutions of different concentrations of both drugs were prepared in the mobile phase. The absorbance of Amlodipine (500µg/ml) and Chlorthalidone (625µg/ml) were recorded at two wavelengths 260nm and 227 nm by using the simultaneous equation method.

Cx = A2ay1- A1ay2/ ax2ay1- ax1ay2 and
CY = A1ax2- A2ax1/ ax2ay1- ax1ay2
Where,
Cx= concentration of Amlodipine
Cy= concentration of Chlorthalidone
ax1and ax2= absorptivity value of Amlodipine 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.

IV. METHOD VALIDATION

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, and LOQ.

Linearity:

Linearity was studied by plotting a graph of absorbance directly proportional to the concentration. A series of standard solutions of Amlodipine concentration range is 40µg/ml to 60 µg/ml and Chlorthalidone was prepared in the concentration range of about 50 µg/ml to 75 µg/ml is shown in below tables (2) & (3). The absorbance values for Amlodipine and chlorthalidone were measured at respective wavelength for each drug separately.

Table No. 2: - Linearity study of Amlodipine

Amlodipine		
% Level	Conc (µg/ml)	Abs
80	40	0.316
90	45	0.356
100	50	0.396
110	55	0.432
120	60	0.476

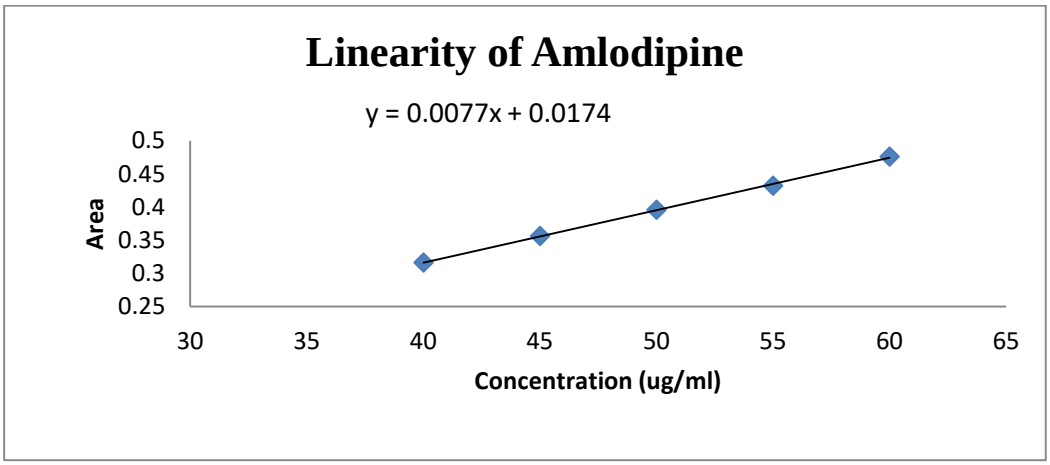

Figure No. 6: - Linearity Graph of Amlodipine

Table No. 3: - Linearity study of Chlorthalidone

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

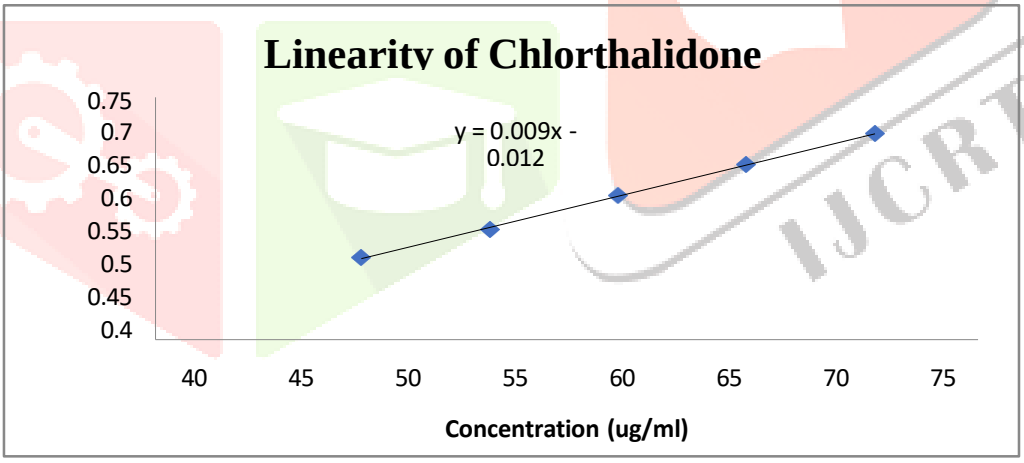

Figure No. 7: - Linearity Graph of Chlorthalidone

Precision:

Six separate solutions comprising concentrations of 45, 50, and 55 µg/ml of Amlodipine and 56.25,62.5, and 68.75 µg/ml of Chlorthalidone were analyzed for repeatability. The absorbance was measured three times each day to determine intra-day and inter-day variation. The %RSD was determined to be less than 2, shown in the below tables (4,5,6, 7).

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

Amlodipine						
Conc (µg/ml)	Absorbance			Avg	STDEV	RSD
	Trial 1	Trial 2	Trial 3			
45	0.319	0.32	0.317	0.318667	0.00	0.48
50	0.394	0.395	0.392	0.393667	0.00	0.39
55	0.445	0.446	0.443	0.444667	0.00	0.34

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

Amlodipine						
Conc (µg/ml)	Absorbance			Avg	STDEV	RSD
	Trial 1	Trial 2	Trial 3			
45	0.318	0.316	0.319	0.318	0.0015	0.48
50	0.392	0.394	0.391	0.392	0.0015	0.39
55	0.442	0.445	0.443	0.443	0.0015	0.34

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

Chlorthalidone						
Conc (µg/ml)	Absorbance			Avg	STDEV	RSD
	Trial 1	Trial 2	Trial 3			
56.25	0.503	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.7: - Inter-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

Accuracy:

This parameter is performed to determine the closeness of the test results with that of the true value which is expressed as % recovery. Recovery studies were carried out at three different levels (80%,100%, and120%) by spiking the same amount of concentration given above in the table for both Amlodipine and Chlorthalidone. Samples were analyzed in Triplicate to calculate % RSD. The % recovery was also calculated below table no. (8 and 9)

Amlodipine			
Std wt. (mg)	% Purity	Std Stock Conc. (µg/ml)	Working Std Area
5	100	50	0.631

Table No. 8: - Recovery Study of Amlodipine

Sample ID	Reps	Spiked Conc. (µg/ml)	Area	Amount Recovered (µg/ml)	% Recovery	AVG	STDEV	% RSD
80%	Rep 1	40.00	0.316	39.85	99.62	99.83	0.364029	0.36
	Rep 2	40.00	0.316	39.85	99.62			
	Rep 3	40.00	0.318	40.10	100.25			
100%	Rep 1	50.00	0.392	49.43	98.87	99.37	0.504414	0.51
	Rep 2	50.00	0.396	49.94	99.87			
	Rep 3	50.00	0.394	49.68	99.37			
120%	Rep 1	60.00	0.476	60.03	100.04	99.48	0.642087	0.65
	Rep 2	60.00	0.47	59.27	98.78			
	Rep 3	60.00	0.474	59.77	99.62			

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

Table No. 9: - Recovery Study of Chlorthalidone

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			

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 Amlodipine and Chlorthalidone.

LOD/ LOQ:

The limit of Quantitation is 3 times more than the Limit of detection respectively. The LOD value of Amlodipine and Chlorthalidone was found to be 3.84 µg/ml and 3.54µg/ml respectively and the LOQ value of Amlodipine and Chlorthalidone were found to be 11.63µg/ml and 10.74µg/ml respectively.
It was calculated for both drugs by using the ANOVA technique.

Table No. 10: - Result of LOD and LOQ

Sr No.	Name of drugs	LOD (µg/ml)	LOQ(µg/ml)
1	Amlodipine	3.84	11.63
2	Chlorthalidone	3.54	10.74

Formula:

$$\text{LOD:} \quad \frac{3.3 \times \text{Std. Error of Intercept}}{\text{Coefficients of X Variable1}}$$

$$\text{LOQ:} \quad \frac{3.3 \times \text{Std. Error of Intercept}}{\text{Coefficients of X Variable1}}$$

V. RESULT AND DISCUSSION:

The proposed method is based on spectrophotometric simultaneous estimation of Amlodipine and Chlorthalidone in this method methanol and distilled water issued as solvent.

Linearity

Linear regression data for the calibration plots revealed good linear relationship between absorbance and concentration over the ranges 40µg/ml to 60 µg/ml of Amlodipine and 50µg/ml to 75µg/ml of Chlorthalidone. The linear equation for the calibration plots was y = 0.0077x + 0.00174 and y = 0.0093x - 0. 012.Regression (R2) being 0.9994 and 0.999for Amlodipine 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 be 99.83% and 99.48% for Amlodipine 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 11.63µg/ml for Amlodipine and 10.74µg/ml and
3.54µg/ml for chlorthalidone. (Table No.10).

VI. CONCLUSION

The proposed method was developed for the determination of Amlodipine 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 Amlodipine and Chlorthalidone in bulk and pharmaceutical dosage form.

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