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Stability Indicating Method Development and Validation for the Simultaneous Estimation of Amlodipine and Chlorthalidone in Bulk and Pharmaceutical Dosage form by RP-HPLC

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ABSTRACT

A simple, rapid and accurate RP-HPLC method was developed to determine the drug such as Amlodipine and chlorthalidone in bulk and pharmaceutical dosage form .The chromatographic separation performed on a shim pack GWS C18(250 mm length x 4.6 mm ,5µ). The eluent were monitored on UV Visible detector at a wavelength of 266nm using mixture of 0.1% formic acid: methanol: acetonitrile in the ratio of (50:5:45v/v) at pH 3 the flow rate 1ml/min. Validation parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantification (LOQ), stability of sample and standard stock solutions and robustness were studies to reported in the ICH guidelines. The retention time of Amlodipine and chlorthalidone was 4.8min, 3.9 min respectively. Assay method further evaluated for Amlodipine and chlorthalidone analysis at low concentration of analyte and found limit of detection 0.78,0.60 µg/mL and limit of quantification is 2.2,1.83 µg/mL respectively. Linearity of Amlodipine and chlorthalidone was from 5-25 µg/mL and 12.5-62.5 µg/mL and the R2 is 0.998. The method was fast, accurate, precise and sensitive hence it can be employed for routine quality control analysis of Amlodipine and chlorthalidone in pure and tablet dosage form.

Keywords: RP-HPLC; Amlodipine; Chlorthalidone; Forced degradation; Validation

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INTRODUCTION

It is used to treat angina, high blood pressure and coronary heart disease. Amlodipine (Figure 1) is chemically described as 3-Ethyl-5-methyl (±)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4 dihydro-6-methyl-3,5-pyridine dicarboxylate, monobenzenesulphonate) is a long acting dihydropyridine(DHP) calcium channel blocker. It works by relaxing blood vessels.

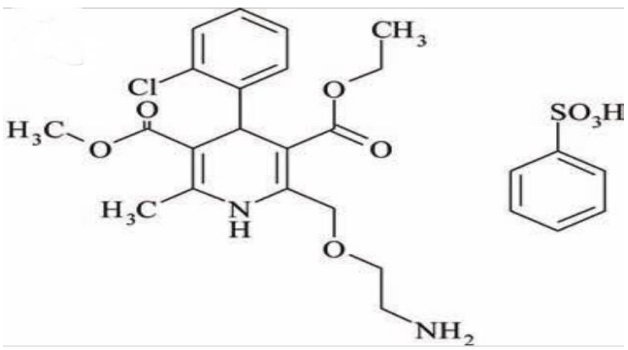

Figure 1: Structure of Amlodipine

Chlorthalidone (Figure 2) is chemically described as (RS)-2-Chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl)benzene-1-sulfonamide. Is a diuretic drug used to treat hypertension. It prevents re absorption of sodium and chloride by inhibiting the Na^+/Cl^- symporter in the distal convoluted tubule [1,2].

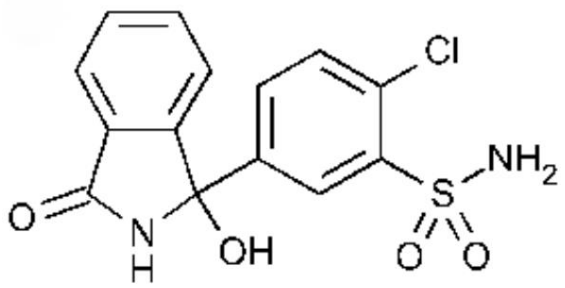

Figure 2: Structure of chlorthalidone

While many methods are available for single or dual combination of the drug under study. The literature survey revealed that several methods are developed for Amlodipine and chlorthalidone individually. Liquid chromatographic methods, liquid chromatography-mass spectrometric method has been developed for Amlodipine and chlorthalidone in pharmaceutical dosage form. To best of our knowledge none of the forced degradation method has been reported for estimation of Amlodipine and chlorthalidone in combined dosage form. Hence, attempt has been making to develop new forced degradation study for its estimation in pharmaceutical dosage formulation with good accuracy, simplicity and sensitivity [3-5].

EXPERIMENTAL SECTION

Materials

Instruments used: Analysis was carried out by Waters 515 HPLC with UV detector, used shim pack GWS C18 (250mm length X 4.6mm,5µ column, shimazdu double beam UV/Visible spectrophotometer model UV1650PC.

Chemicals and reagents: Active pharmaceutical ingredients Amlodipine and chlorthalidone were obtained as a gift sample from Sai Moheshwar Healthcare, Vadodara. The pharmaceutical dosage form (Amlodac CH (5 mg/12.5 mg) was purchased from local pharmacy. HPLC grade acetonitrile was purchased from Himedia laboratories Pvt ltd, Mumbai, India. Formic acid was purchased from sigma Aldrich.

Method Development

Preparation of standard stock solution: Accurately weigh and transferred 5 mg of Amlodipine and 12.5 mg of chlorthalidone in 10ml volumetric flask and make up to 10 mL with diluents. 5 mL from stock solution were taken and transferred into 50ml volumetric flask make up with 50ml diluents. 1ml from above two stock solutions was taken into a 10 mL volumetric flask and made up to 10 mL diluents [6].

Preparation of sample stock solution: 10 tablets are weighed and powdered. The weight 150mg is equivalent to 5mg of Amlodipine and 12.5 mg of chlorthalidone was transferred into 10 mL volumetric flask and make up with 10ml diluents. 5ml from stock solution were taken and transferred into 50ml volumetric flask and make up with 50 mL diluents. 1 mL from above stock solution was taken and transferred into 10 mL volumetric flask make up to 10 mL with diluents [7-10].

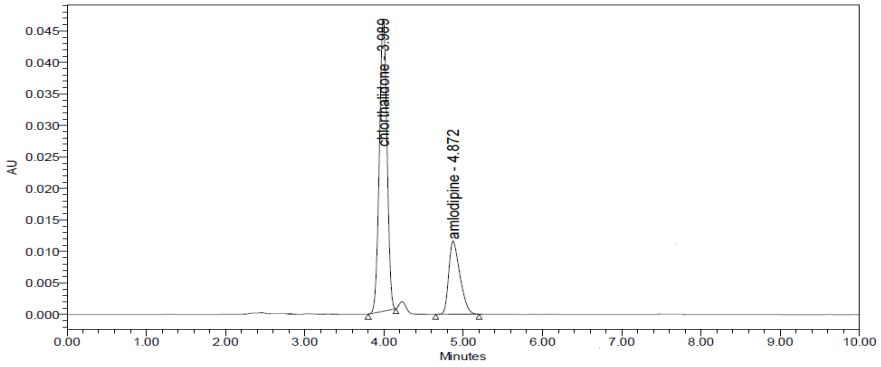

Figure 3: Chromatogram of sample (Formulation)

Method Validation

The method was validated according to ICH guidelines. The different validation parameters are performed like linearity, precision, accuracy, limit of detection, limit of quantification, robustness (Figure 3).

Linearity: Linearity was evaluated by analysis of preparing the solution range of Amlodipine (5-25 µg/mL) and chlorthalidone (12.5-62.5 µg/mL). The linearity was plotted against peak area Vs concentration as explained in Figures 4 and 5. The correlation co efficient was found to be 0.998 respectively [11-13] (Table 1).

Table 1: Linearity of amlodipine and chlorthalidone

Sample no	Concentration(µg/mL)		Peak area	
	Amlodipine	Chlorthalidone	Amlodipine	Chlorthalidone
1	5	12.5	39219	110819
2	10	25	80350	226873
3	15	37.5	114381	320610
4	20	50	160984	447067
5	25	62.5	200266	557858
Correlation coefficient			0.998	0.998

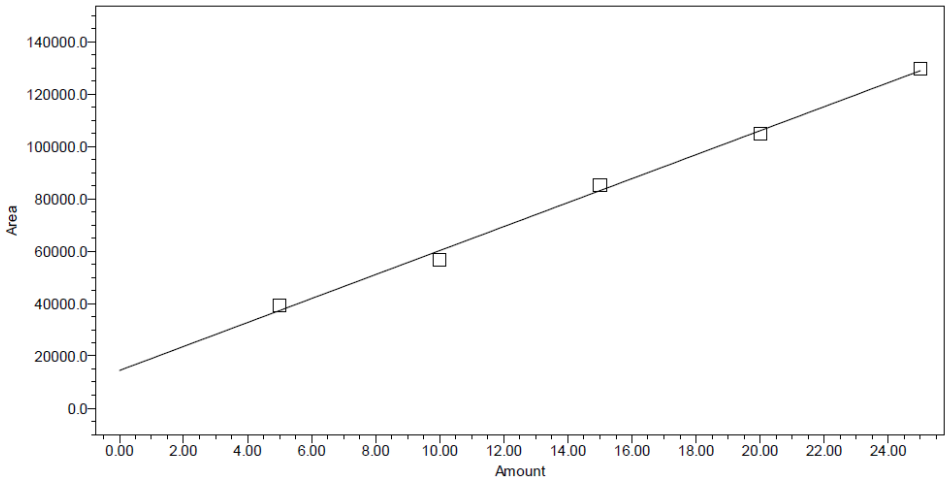

Figure 4: Linearity of Amlodipine

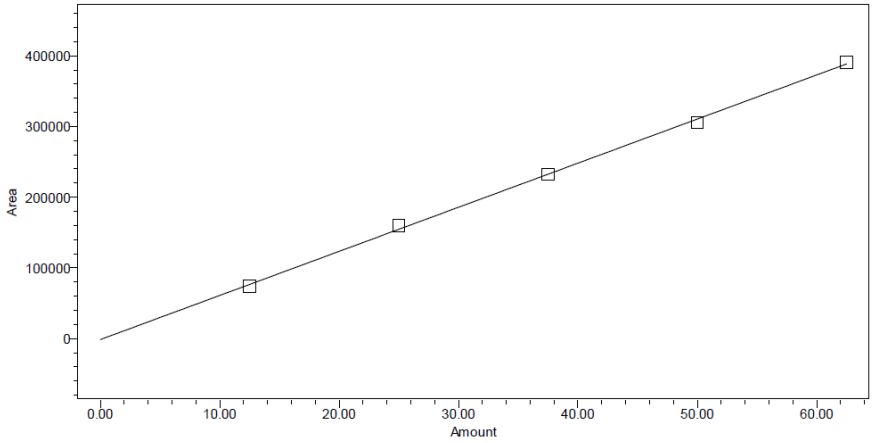

Figure 5: Linearity of chlorthalidone

Accuracy: The accuracy study was performed for 50%, 100%, 150% for Amlodipine and chlorthalidone by three replicate analysis of the sample containing pharmaceutical dosage form were calculated in Table 2 and Table 3.

Table 2: Recovery studies of amlodipine

Nominal concentration (µg/mL)	LQC		MQC		HQC	
	7.5		15		22.5	
S.NO	Calculated concentration (µg/mL)	Accuracy %	Calculated concentration (µg/mL)	Accuracy %	Calculated concentration (µg/mL)	Accuracy %
1.	7.47	99.6	14.79	98.6	22.18	98.5
2.	7.34	97.8	14.99	99.9	22.44	99.8
3.	7.48	99.7	14.72	98.1	22.47	99.8
4.	7.29	97.2	14.77	98.5	22.48	99.9
5.	7.31	97.4	14.81	98.7	22.42	99.6
6.	7.35	98.1	14.62	99.5	22.43	99.7
Mean		98.3		98.8		99.5
SD		1.091		0.676		0.524
%RSD		1.11		0.68		0.52

Table 3: Recovery studies of chlorthalidone

Nominal concentration (µg/mL)	LQC		MQC		HQC	
	18.75		37.5		56.25	
S.NO	Calculated concentration (µg/mL)	Accuracy %	Calculated concentration (µg/mL)	Accuracy %	Calculated concentration (µg/mL)	Accuracy %
1.	18.71	99.8	37.46	99.9	56.22	99.9
2.	18.73	99.9	37.43	99.4	55.99	99.5
3.	18.61	99.3	37.31	99.5	56.17	99.8
4.	18.72	99.8	37.38	99.6	56.10	99.8
5.	18.68	99.6	37.44	99.8	56.13	99.7
6.	18.65	99.4	37.39	99.5	56.20	99.9
Mean		99.6		99.6		99.7
SD		0.242		0.209		0.150
%RSD		0.24		0.21		0.15

Precision: In repeatability, six standard solutions were prepared each having a concentration of 15 µg/mL and 37.5 µg/mL of Amlodipine and chlorthalidone. The response of each of these solutions was measured and percentage relative standard deviation (%RSD) was calculated in Tables 4 and 5.

Table 4: Interday and intraday precision data for amlodipine

Concentration µg/mL	Peak area		%RSD	
	Intraday	Interday	Intraday	Interday
10	77763 78832 78945	77762 78246 78687	0.830	0.590
15	123843 124543 125756	123845 124446 125385	0.780	0.623
20	196654 196865 197685	196644 197080 198808	0.276	0.577

Table 5: Interday and intraday precision data for chlorthalidone

Concentration µg/mL	Peak area		%RSD	
	Intraday	Interday	Intraday	Interday
25	185092 186284 187394	185093 187063 187654	0.618	0.718
37.5	292970 295992 298030	292969 293800 297387	0.861	0.796
50	376576 378567 379897	376575 377875 379885	0.441	0.146

Limit of detection and limit of quantification: The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were established by injecting the low concentration of the standard solution using the developed RP-HPLC method. The limit of detection found to be 0.78 µg/mL and 0.60 µg/mL, respectively. The limit of quantification was found to be 2.21 µg/mL and 1.83 µg/mL, respectively.

Robustness: The robustness of test method was demonstrated by carrying out mobile phase variation ± 2%, flow rate variation ±2% and wavelength variation ±2% is shown in Table 6.

Table 6: Robustness

		Amlodipine %RSD	Chlorthalidone %RSD
Flow rate	0.8ml/min	1.4	1.3
	1.2ml/min	0.9	1.0
Mobile phase	43:5:52	0.9	1.2
	48:5:47	0.7	0.9
wavelength	264nm	o.4	0.7
	268nm	0.8	1.1

Forced Degradation Studies

Forced degradation studies were performed on Amlodipine and chlorthalidone. The drug was subjected to under various stress conditions like acid degradation (0.1 M HCl), base degradation (0.1 M NaoH), oxidation30% ($H_2O_{2)}$, thermal and photolytic (exposure to light).

Acid degradation: Accurately weigh and transferred 5.mg of Amlodipine and 12.5 mg of chlorthalidone in 10ml volumetric flask to add 0.1M HCl and make up the volume with mobile phase kept aside for 3.hrs. After completion of 3 hrs, about 0.1ml of the above solution was transferred into 10ml volumetric flask and diluted with10 mL using mobile phase (5. µg/mL of AMLO, 12.5. µg/mL of CHLOR). The solution was injected into HPLC and chromatogram was recorded (Figure 6).

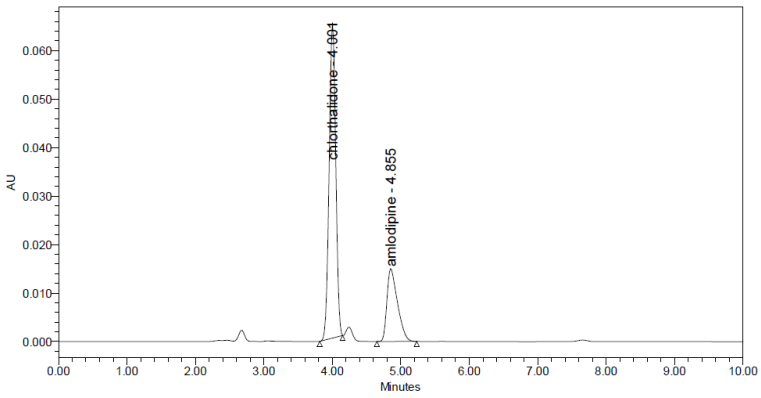

Figure 6: Chromatogram of acid degradation

Alkali degradation studies: Accurately weigh and transferred 5 mg of Amlodipine and 12.5 mg of chlorthalidone in 10 mL volumetric flask to add 0.1 M NaoH and make up the volume with mobile phase kept

aside for 3 hrs. After completion of 3 hrs, about 0.1ml of the above solution was transferred into 10 mL volumetric flask and diluted with10 mL using mobile phase (5 µg/mL of AMLO, 12.5 µg/mL of CHLOR). The solution was injected into HPLC and chromatogram was recorded (Figure 7).

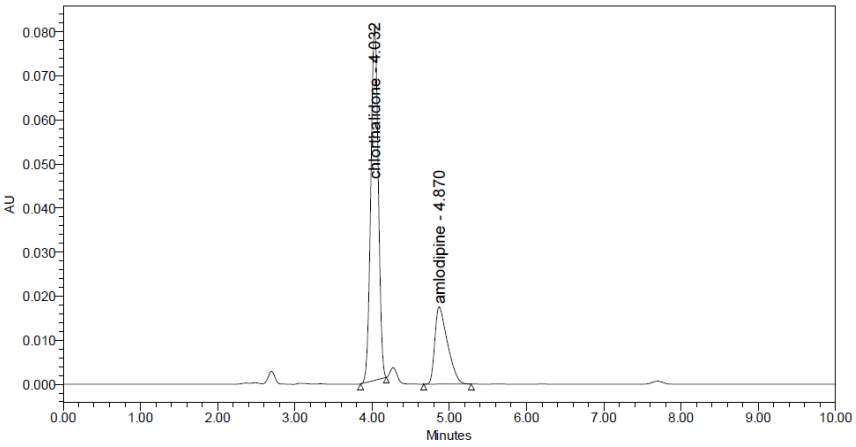

Figure 7: Chromatogram of acid degradation

Oxidation condition: Accurately weigh and transferred 5 mg of Amlodipine and 12.5 mg of chlorthalidone in 10 mL volumetric flask to add 30% H_2O_2 and make up the volume with mobile phase kept aside for 3 hrs. After completion of 3 hrs, about 0.1ml of the above solution was transferred into 10ml volumetric flask and diluted with10 mL using mobile phase (5 µg/mL of AMLO, 12.5 µg/mL of CHLOR). The solution was injected into HPLC and chromatogram was recorded (Figure 8).

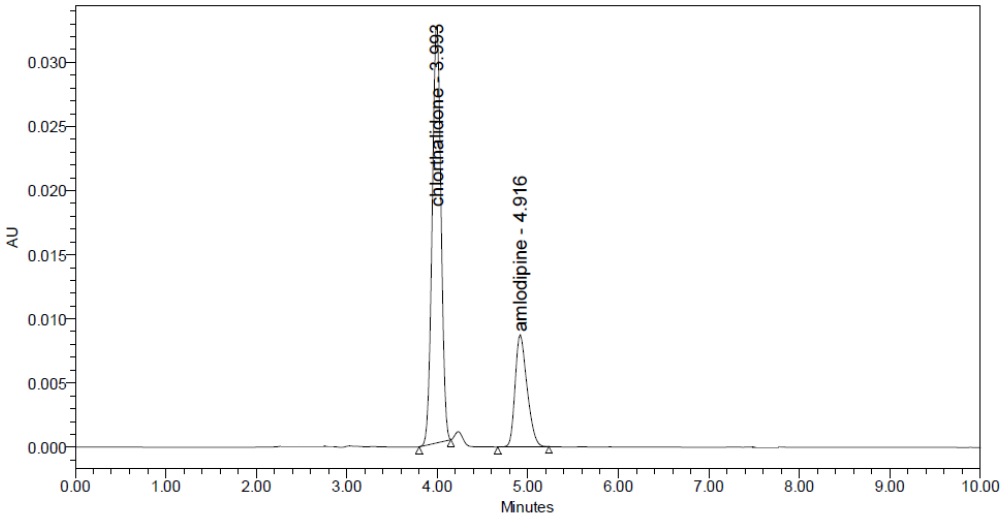

Figure 8: Chromatogram of oxidation condition

Thermal degradation: Accurately weighed 5 mg of Amlodipine and 12.5 mg of chlorthalidone was transferred into a clean, dry petridish. The petridish was placed in an oven at 50˚C for 3hrs. The drug was transferred into 10 mL volumetric flask and make up the volume with mobile phase. 0.1ml of the above solution was transferred into 10ml volumetric flask and diluted with10ml using mobile phase (5 µg/mL of AMLO, 12.5 µg/mL of CHLOR). The solution was injected into HPLC and chromatogram was recorded (Figure 9) [14-16].

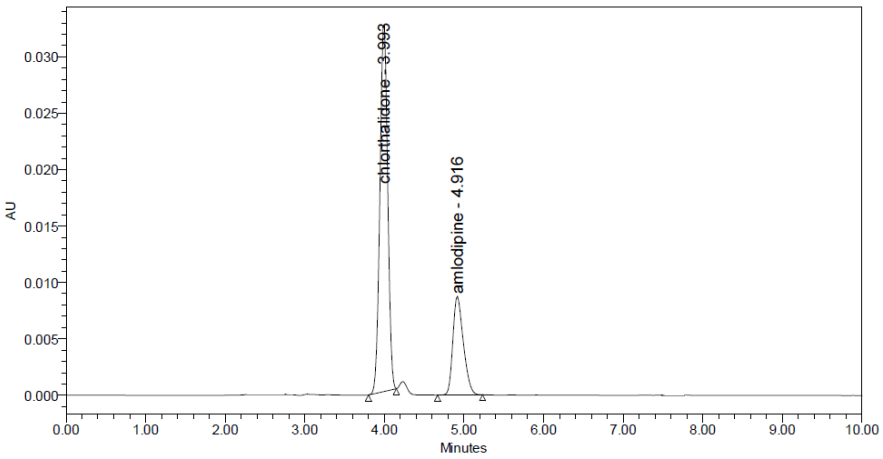

Figure 9: Chromatogram of thermal degradation

Photolytic degradation: Accurately weighed 5 mg of Amlodipine and 12.5 mg of chlorthalidone was transferred into a clean, dry petridish. The petridish was placed in direct sunlight for 5days. The drug was transferred into 10ml volumetric flask and make up the volume with mobile phase. 0.1ml of the above solution was transferred into 10 mL volumetric flask and diluted with10 mL using mobile phase (5 µg/mL of AMLO, 12.5 µg/mL of CHLOR). The solution was injected into HPLC and chromatogram was recorded (Figure 10).

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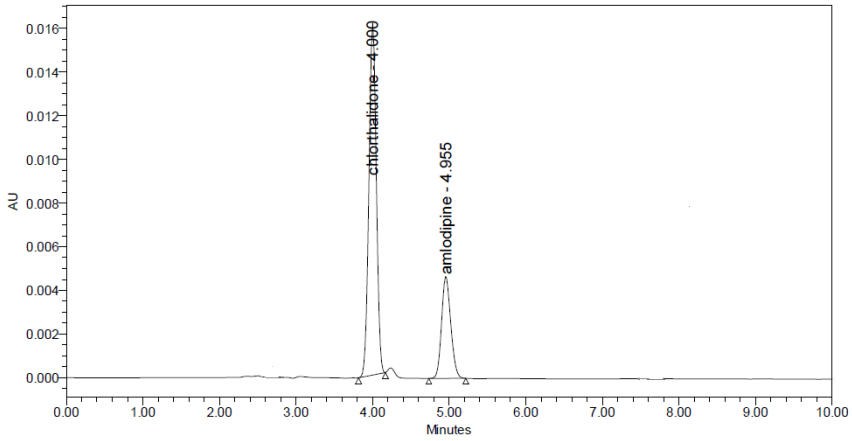

Figure 10: Chromatogram of photolytic degradation

RESULTS AND DISCUSSION

In present work an RP-HPLC method was developed for the simultaneous estimation of Amlodipine and chlorthalidone in tablet dosage form using UV detector. The chromatographic conditions were optimized to provide appropriate results of the assay which will be able to estimate the drugs in the formulation within single wavelength using simple mobile phase and appropriate flow rate. The selection of mobile phase was based upon the polarity. Different trials were performed and the chromatographic conditions were fixed. Acetonitrile: methanol:0.1%formic acid (45:5:50%v/v) pH 3 at 1 mL flow rate using shim pack GWS C18 (250 mm length X 4.6 mm,5 µ) column (Table 7).

Table 7: Chromatographic conditions

HPLC System	**Waters 515**
Column	shim pack GWS C18, 5µm (250 mm X 4.6 mm)
Mobile phase	Acetonitrile:methanol:0.1%formic acid (45:5:50%v/v)
Flow rate	1 mL/min
Injection volume	20 µL/min
Run time	10 mins
Detector	UV Detector

The retention time was 3.9 min for chlorthalidone and 4.8 min for Amlodipine. The linearity was found to be in the range of Amlodipine 5-25µ/ml and chlorthalidone 12.5-62.5 µg/mL. The correlation co efficient was 0.998.The method was validated as per ICH guidelines by using various parameters to ensure the accomplishment of its application (Tables 8 and 9).

Table 8: System suitability test parameters

S.NO	Parameters	Amlodipine	Chlorthalidone
1	Range	5-25 µg/mL	12.5-62.5 µg/mL
2	Detection Wavelength	266nm	266nm
3	Tailing Factor	1.1	1.3
4	Retention Time	4.8	3.9

Table 9: Assay of formulation

Drug	Amount labelled(mg/mL)	Amount found (mg/mL)	%assay	%RSD
Amlodipine	5 mg	4.8	97.1	1.7
Chlorthalidone	12.5 mg	12.31	98.4	0.48

Forced degradation study

The stability studies were conducted by exposing the dosage form to different stress conditions like acid, base, peroxide, thermal and light. It was found that dosage form slightly degraded in acid, base, oxidation conditions but stable on thermal and photolytic condition (Tables 10-12).

Table 10: Forced degradation studies of amlodipine

Stress condition	Sample concentration	Area	% Assay	% Degradation
Acid	5 µg/mL	42421	92.4	7.6
Base	5 µg/mL	42300	92.7	7.3
Oxidation	5 µg/mL	41987	93.4	6.6
Photolytic	5 µg/mL	41678	94.1	5.9
Thermal	5 µg/mL	41768	93.8	6.1

Table 11: Forced degradation studies of chlorthalidone

Stress condition	Sample concentration	Area	% assay	%degradation
Acid	12.5 µg/mL	121321	91.3	8.7
Base	12.5 µg/mL	119497	92.7	7.3
Oxidation	12.5 µg/mL	118456	93.3	6.7
Photolytic	12.5 µg/mL	117652	94.1	5.9
Thermal	12.5 µg/mL	116653	94.9	5.1

Table 12: Study on degradation

Stress condition	Sample concentration	% Assay		% Degradation	
		AMLO	CHLOR	AMLO	CHLOR
Acid	100 µg/mL	92.7	91.7	7.3	8.3
Base	100 µg/mL	93.3	92.9	6.7	7.1
Oxidation	100	93.9	93.7	6.1	6.3
Photolytic	100 µg/mL	94.3	94.6	5.7	5.4
Thermal	100 µg/mL	94.7	94.9	5.3	5.1

CONCLUSION

A simple, novel, precise and economical stability indicating RP-HPLC method for the simultaneous estimation of Amlodipine and chlorthalidone. The method was subjected to forced degradation studies and the percentage degradation at each degradation study was within the limits. The results of each validation parameter were in good with acceptance criteria. Hence, this method can be applied for the estimation of Amlodipine and chlorthalidone in drug testing laboratories and pharmaceutical industries.

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