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HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY OF SIMULTANEOUS ESTIMATON OF CILNIDIPINEHYDROCHLORIDE AND CHLORTHALIDONE IN ITS COMBINEDDOSAGE FORMS

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ABSTRACT

Hypertension is a major health problem throughout the world because of its high prevalenceand its association with increased risk of cardiovascular disease. High blood pressure (BP) or HTN has been recognized by the world health organization (WHO) as the most important preventable cause of premature morbidity and mortality in developed countries. HTN is a condition which probably requires lifelong treatment and life style modifications. The HPTLC instrument used in this purpose having a twin trough chamber, Camage UV-TLC plate scanner, video densitometer. The mobile phase was a combination of Ethyl acetate :Hexane in the ratio of 6:4 v/v stationary phase was HPTLC recoated plate of Silica gel G60F254.Scanning wavelength was 235 nm. The Rf values were obtained as 0.98 for Cilnidipine and 0.77 for Chlorthalidone. Assay result was found to be 101.40%w/w for Cilnidipine and 99.94%w/w for Chlorthalidone (Area wise). Limit of detection and Limit of Quantification values were obtained as 0.3956 ng and 1.1980ng (Area wise) respectively.

KEYWORDS: Hypertension, High blood pressure (BP), UV-TLC.

INTRODUCTION

Hypertension is defined as either a sustained systolic BP of greater than 140 mm Hgor a sustained diastolic BP of greater than 90 mm Hg. Hypertension is known as a silent killer, although many of these individuals have no symptoms, chronic hypertension either systolic or diastolic can lead to serious health problems. The incidence of morbidity and mortality significantly decreases when hypertension is diagnosed early and is properly treated. Numerous medicines are introduced into the market every year. So, it is important to evaluate thequality and efficacy of these medicines. The complex task of pharmaceutical analysis include development of new pharmacopeial methods, stress testing to validate stability-indicating methods, impurity analysis and identification, herbal or animal material analysis, cleaning validation, degradation test and stability study.

HIGH PERFROMANCE THIN LAYER CHROMATOGRAHY

The basic principle of Thin Layer Chromatography is adsorption. The major components of TLC are a stationary phase and a mobile phase. The stationary phase isa sheet of glass, plastic or aluminum foil, which is coated with a thin layer of adsorbent usually silica gel, aluminum oxide or cellulose. After the application of the sample on the stationary phase, the mobile phase is allowed to move through the stationary phase via capillary action. The separation of the components in the sample takes place depended on the affinity of the components towards the stationary phase and mobile phase. Organic solvent or mixture of solvents is used as mobile phase to achieve a good resolution. High performance thin layer chromatography (HPTLC) is a sophisticated instrumental technique based in the full capabilities of thin layer chromatography. Theadvantages of automation, scanning, full optimization, selective detection principle, minimum sample preparation, hyphenation, etc. enable it to be a powerful analytical tool for chromatographic information of complex mixtures of inorganic, organic, and biomolecules. The term HPTLC is used for the technique in which substances are accurately and precisely assayed using high performance grade silica gel. In HPTLC, the sorbent material like silica gel G60 has finer particle size distribution than conventional TLC material. It is a powerful, reliable and cost-effective method for qualitative and quantitative analysis. In HPTLC, the mobile phase moves through the pre-coated stationary phase by capillary action or by gravity.

The position of any solute spot in TLC is characterized by its retention/retardation factor Rf. It is a fundamental qualitative value and is expressedas:

$$Rf = \frac{\textbf{distance travelled by solute from application line}}{\textbf{distance travelled by solvent from application line}}$$

Rf values range from 1.0 for analyte migrating to the solvent front to 0.0 for analyte strongly retained at the point of application. The reproducibility of Rf value depends on many factors, such as quality of the sorbent, humidity, layer thickness, development distance and ambient temperature. Overloading of sample usually resultsin slight increase in Rf value.

DRUG PROFILE

1. CILNIDIPINE HYDROCHLORIDE

Drug category: Antihypertensive

Structure

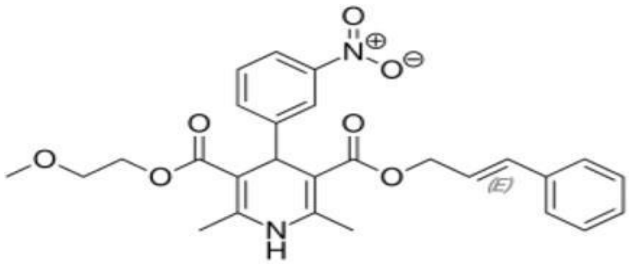


Figure 1: Structure of cilnidipine hydrochloride.

Cilnidipine is a dihydropyridine calcium channel blocker used in the treatment ofhypertension.

Table 1: Description of Cilnidipine hydrochloride.

Brand name	**Cilacar, cinod**
IUPAC Name	3-(E)-3-Phenyl – 2-propenyl 5,2 Methoxy ethyl 2,6 – dimethyl - 4 - (m- nitro phenyl) 1,4 – dihydropyridine -3,5 - dicarboxylate.
Molecular formula	$C_{27}H_{28}N_2O_7$
Molecular weight	492.52 gm/mole

Table 2: Physiochemical properties of Cilnidipine hydrochloride.

Appearance	Light yellowish solid
Solubility	Soluble in methanol, Ethanol, Acetonitrile and insoluble in water
Melting point	110° C
$_pKa$	2.5 – 2.6

PHARMACODYNAMICS

Mechanism of action

Cilnidipine is a dihydro pyridine calcium channel blocker. It is an L- type and N- type calcium channel blocking function. It inhibits cellular calcium influx, thus causing vasodilation. Cilnidipine has greater selectivity for vascular smooth muscle. It has null action at the SA or AV nodes and negative inotropic activity is poorly seen at therapeutic doses.

Therapeutic efficacy / Indications: Hypertension

Adverse reactions

Dizziness, headache, flushing, peripheral oedema, tachycardia, GI disturbances, increased micturition frequency, lethargy, eye pain, depression, hypotension, ischemic chest pain, cerebral or myocardial ischemia, transient blindness, abnormal liver function, palpitation rashes, fever, gingival hyperplasia, myalgia, tremor, impotence.

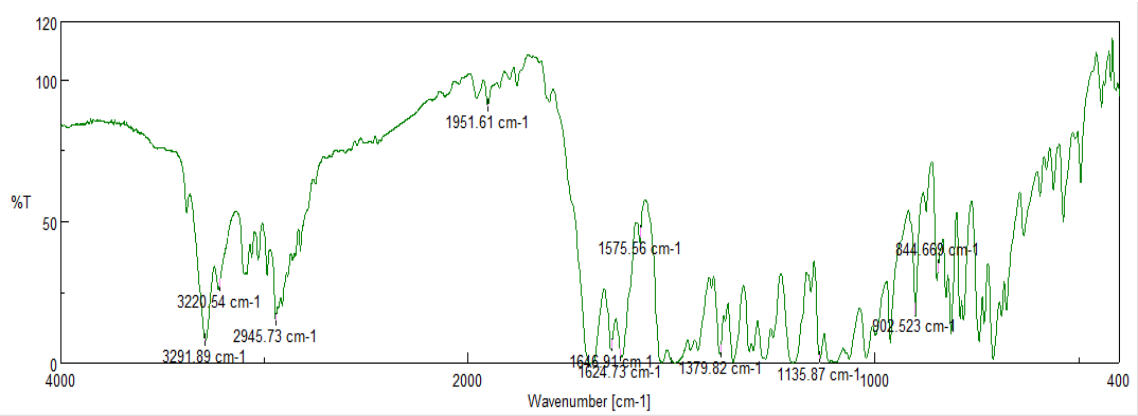


Figure 1: FTIR spectrum of cilnidipine hydrochloride.

PHARMACOKINETICS

Absorption: Oral absorption is found to be 65 %

Distribution: Volume of distribution 3-5 L/kg and plasma protein binding is 75 %

Metabolism: Pre – systemic metabolism is noted to be 0.5 %

Excretion: Excreted unchanged by the kidneys.

CONTRAINDICATIONS

Carcinogenic shock, recent MI or acute unstable angina, Severe aortic stenosis.

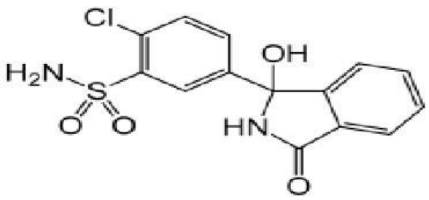
DRUG INTERACTIONS

Carbamazepine, aldesleukin, phenytoin, antipsychotics that cause hypotension, may modify insulin and glucose responses, quinidine, rifampicin, cimetidine, erythromycin and other antihypertensive.

2. CHLORTHALIDONE

Drug category: Diuretics

Structure



It is along acting thiazide – like antihypertensive diuretic used in the treatment of oedema associated with congestive heart failure.

Table 3: Description of chlorthalidone.

Brand name	CTD
IUPAC name	2- chloro – 5 – (1 – hydroxy – 3 – oxo -2,3 – dihydro -1H – isoindol -1-yl) benzene -1- sulphonamide
Molecular formula	$C_{14}H_{11}ClN_2O_4S$
Molecular weight	338.76 g

Table 4: Physicochemical properties of chlorthalidone.

Appearance	White to yellowish white crystalline powder
Solubility	Practically insoluble in water, in etherand in chloroform, soluble in methanol, slightly soluble in alcohol.
Melting point	218-264°C
$_p$Ka	9.4

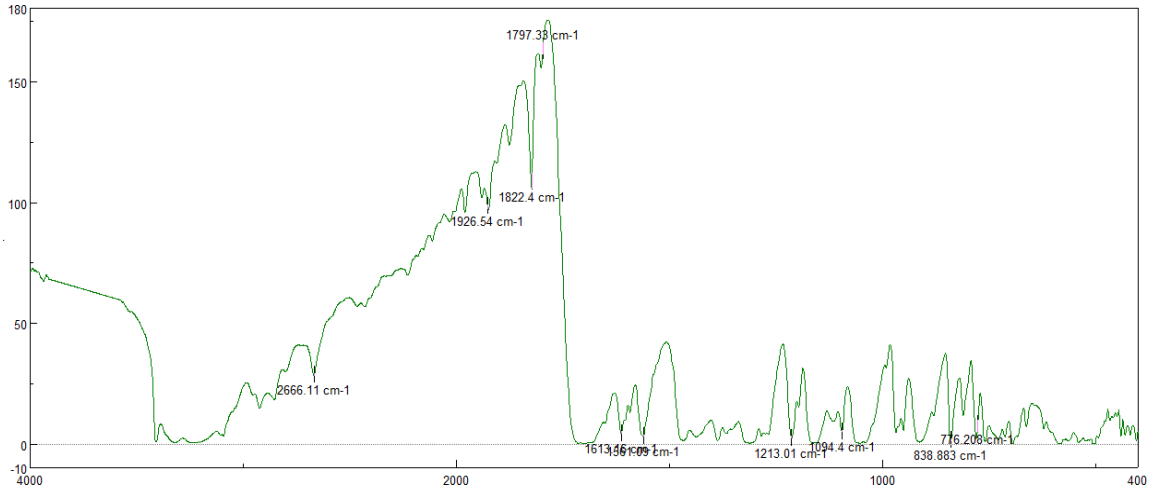


Figure 2: FTIR Spectrum of chlorthalidone.

PHARMACODYNAMICS

Mechanism of action

Chlorthalidone is a diuretic drug, that inhibit sodium ion transport across the renal tubular epithelium in the cortical diluting segment of the ascending loop of Henle. By increasing the delivery of sodium to the distal renal tubule, chlorthalidone indirectlyincreases potassium exchange mechanisms. It has a longer duration of action.

Indications

For the management of hypertension either as the sole therapeutic agent or to enhance the effect of other antihypertensive drugs in the more severe forms of hypertension.

Adverse reactions

- Sore throat with fever
- Unusual bleeding
- Severe skin rash with peeling skin
- Difficulty swallowing or breathing

PHARMACOKINETICS

Absorption: Oral absorption is found to be 65%.

Distribution: Volume of distribution 3 – 5 L/kg and plasma protein binding is 75 %.

Metabolism: Pre – systemic metabolism is noted to be 0.5%.

Excretion: Excreted unchanged by the kidneys.

CONTRAINDICATIONS

Hypersensitivity to thiazide, related diuretics or sulphonamide – derived drugs, anuria etc.

DRUG INTERACTIONS

Chlorthalidone may added to potentiate the action of other antihypertensive drugs. Insulin requirements in diabetic patients may change. Increased responsiveness to tubocurarine. Decrease arterial responsiveness to norepinephrine. Increase the risk of lithium toxicity.

METHOD

Reagents and chemicals

- Cilnidipine hydrochloride RS
- Chlorthalidone RS.
- Methanol HPLC grade from Merck specialties (p) Ltd Mumbai.
- Ethyl acetate HPLC grade from Merck specialties (P) Ltd. Mumbai
- Hexane HPLC grade from Merck specialties (P) Ltd. Mumbai
- Commercially available Cilnidipine hydrochloride and Chlorthalidone (Cilacar – C containing 10mg of cilnidipine and 6.25mg of chlorthalidone) marketedby J. B. Chemicals and pharmaceuticals Ltd.

Equipment

- Shimadzu analytical balance (Model ATX224)
- GT sonic, professional ultrasonic cleaner
- Application mode: CAMAG Linomat IV
- Development mode: CAMAG Twin trough chamber
- Scanner: TLC Scanner III
- Visualization: CAMAG UV TLC plate view cabinet
- Quantification: CAMAG Video Densitometer
- Stationary phase: TLC plates with 250µm thickness; E. Merck, Darmstadt, Germany

Twin trough chamber **TLC spotter** **TLC Scanner**

Figure 3: Instruments used in HPTLC.

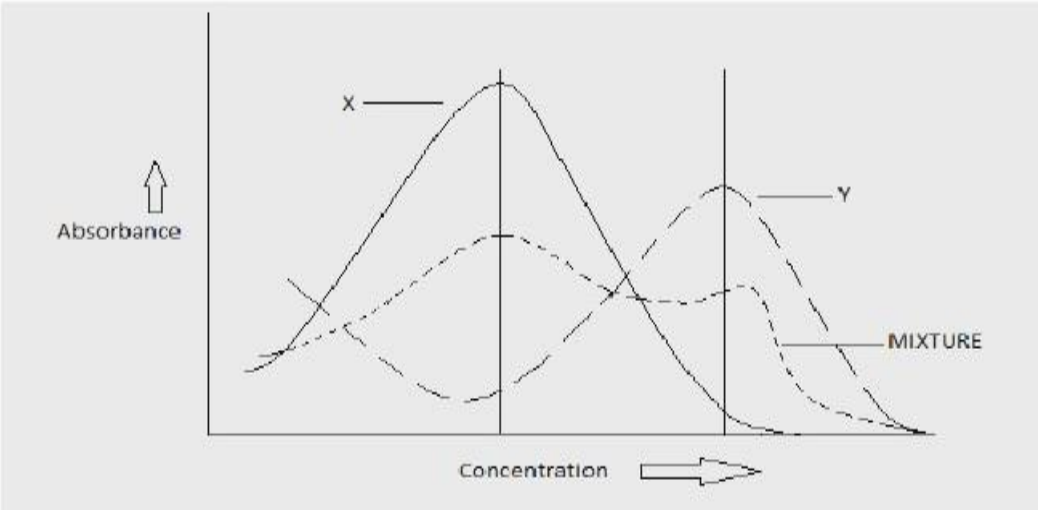


Figure 4: Absorption of two single components and mixture in UV region.

Methodology adopted

Preparation of standard solution of Cilnidipine hydrochloride and Chlorthalidone.

Stock solution of Cilnidipine hydrochloride RS in methanol

Weighed accurately 10 mg of cilnidipine hydrochloride RS and transferred into a 10 mL standard flask. Added about 7mL methanol, sonicated for 10 minutes and then made up to the volume using methanol. This solution had a concentration of 1000µg/ml.

Stock solution of Chlorthalidone in methanol

Weighed accurately 10mg of chlorthalidone RS and transferred into a 10ml standard flask and made up the volume after sonication for 10 min. This solution had aconcentration of 1000 µg/ml.

Preparation of standard drug mixture

10mg of cilnidipine hydrochloride RS and 6.25 mg of chlorthalidone RS were weighed separately and transferred to a 10 ml standard flask The drug mixture was allowed to dissolve in sufficient quantity of methanol by shaking for 15 min and the volume was made up to the mark with methanol to obtain a mixture with a concentration of 1000µg/ml of cilnidipine hydrochloride and 625µg/ml of chlorthalidone (solution A).

Development of solvent system

The mobile phase was selected based on the polarity of analyte and adsorption property of silica gel plates. The solubility of the drug played a significant role in the selection of mobile phase. The suitable solvent system was selected by a series of trialand error process. Different solvent systems were used in different proportions andthe summary is listed in the table below.

Table 6: Solvent System –trial and error data.

Sl. No	Solvent system	Ratio	Inference
1	Methanol		Solvent front
2	Methanol: Chloroform	-	Spot moved up to solvent front
3	Toluene	-	Spot moved with low resolution
4	Chloroform: Toluene	4:6	Spot moved with little resolution
5	Methanol: Cyclohexane	5:5	Spot moved up to solvent front
6	Ethyl acetate: Toluene	7:3	Acceptable resolution
7	Ethyl acetate: Hexane	6:4	Good resolution

Development of chromatogram

Selection of chromatographic layer.

HPTLC pre-coated plates of silica gel G60F254 were employed for the spotting of standard solutions.

Preparation of mobile phase and saturation of Twin trough chamber

Mobile phase (ethyl acetate and hexane the ratio 6:4 v/v) was freshly prepared and transferred into a clean and dried twin trough chamber. The chamber was then allowed to saturate for 30 minutes.

Activation of plate and sample application

A single track was selected on the activated pre-coated HPTLC plate and spotting was done by using CAMAG Linomat IV sample applicator in the form of bands. Cilnidipine hydrochloride standard was applied on the first track and chlorthalidone on the second track. Volume of the sample application was selected according to the volatility of the solvent used for preparing the sample solution. Concentration was selected as 1000 µg/mL by trial-and-error method. The appliedband was sharp when the volume was 10 µL. A band width of 4 mm was selected for the entire experiment. The following manual adjustments were done in the Linomat applicator.

Plate size : 20x10 cm

Start position : 10 mm

Band width : 4 mm Application volume: 10µL Flow rate : 10 µL/sec

After application, the plate was taken out and the position of the spot wasvisualized and confirmed under the UV cabinet at 254 nm.

Development of spot

The plate was developed in the saturated twin trough chamber containing the mobile phase. The plate was dried after development and viewed under UV cabinet to evaluate the spots obtained.

Determination of Rf value of Cilnidipine hydrochloride and chlorthalidone

Detection and visualization

The developed plate was mounted on the CAMAG HPTLC scanner III and scanned from 200 to 400 nm. The spot showed good response at 235 nm. The Rf values are furnished in the table.

Table 7: Retention time of Cilnidipine and Chlorthalidone.

Drug	Ret. time
Cilnidipine hydrochloride	0.98
Chlorthalidone	0.77

Preparation of calibration curves of cilnidipine hydrochloride and chlorthalidone and analysis of combined tablet dosage form

Preparation of standard solution of cilnidipine hydrochloride RS in methanol

Weighed accurately 100mg of cilnidipine hydrochloride and transferred to 100ml standard flask containing HPLC grade methanol and made up the volume. Thissolution had a concentration of 1000 µg/ml. From the above solution 1µl, 3µl, 5µl, 7 and 10µl were taken.

Preparation of standard solution of chlorthalidone RS in methanol

Weighed accurately 100mg of chlorthalidone RS and transferred to a 100ml standard flask and dissolved in methanol and made up the volume. This had a concentration of 1000µg/ml. From the above solution 1µl,3µl,5µl,7and 10µl were taken.

Preparation of standard drug mixture

10 mg of cilnidipine hydrochloride and 6.25 mg of chlorthalidone RS were weighed separately and transferred to a 10 ml standard flask. The drug mixture was allowed to dissolve in sufficient quantity of methanol by shaking for 15 min and the volume was made up to the mark with methanol to obtain a mixture with a concentration of 1000µg/ml of cilnidipine hydrochloride, 625µg/ml of chlorthalidone.

Preparation of sample solution

Details of Analysed dosage form

Trade name : Cilacar – C 10
Each tablet contains : Cilnidipine 10mg and chlorthalidone 6.25 mg
Batch No. : ABJ8005
Mfg. Date : AUG.18
Exp. Date : JUL.21
Mfd. By : J.B CHEMICALS & PHARMACEUTICALS

Twenty tablets of Cilacar - C were taken and its contents separated and weighed. Average weight was determined. A quantity of powder equivalent to 10mg of cilnidipine and 6.25 mg of chlorthalidone was accurately weighed and transferred to a stoppered flask and extracted with methanol by shaking for 15 minutes. The solution was transferred to a 10ml standard flask through a Whatman No.1 filter paper. The resulting solution had concentration of 1000µg/ml of cilnidipine and 6.25 µg/ml of chlorthalidone as per label claim

Development of chromatogram and selection of chromatographic layer

HPTLC pre-coated plates of silica gel G 60 F254 were employed for the spottingof standard solutions. Preparation of mobile phase and saturation of twin trough chamber. Mobile phase containing ethyl acetate and hexane (6:4) was freshly prepared and transferred to a clean twin trough chamber. The chamber was then allowed to saturate for 30 minutes. Activation of plates and sample application**.**12 tracks were selected on the activated pre-coated HPTLC plate and spotting was done by using CAMAG Linomat IV automatic sample applicator in the form of bands. Cilnidipine hydrochloride standard were applied on the first five tracks and chlorthalidone on the next five tracks. Standard drug mixture and sample were appliedon another plate. After application the plate was taken out and the position of the spots were visualized and confirmed under UV cabinet at 254 nm.

Development of spots: -The plate was developed in the saturated twin trough chamber containing the mobile phase. The plate was dried after development and viewed under UV lamp to evaluate the spots obtained. The spots were uniform without tailing.

Scanning and integration of chromatogram

The developed plate was mounted on the CAMAG HPTLC Scanner IV and scanned at 235 nm. The results are furnished in the table below. The calibration plots of concentration v/s peak height and concentration v/s peak area, overlay spectrum, plate developed chromatograms of standards and samples were given respectively in the figures below.

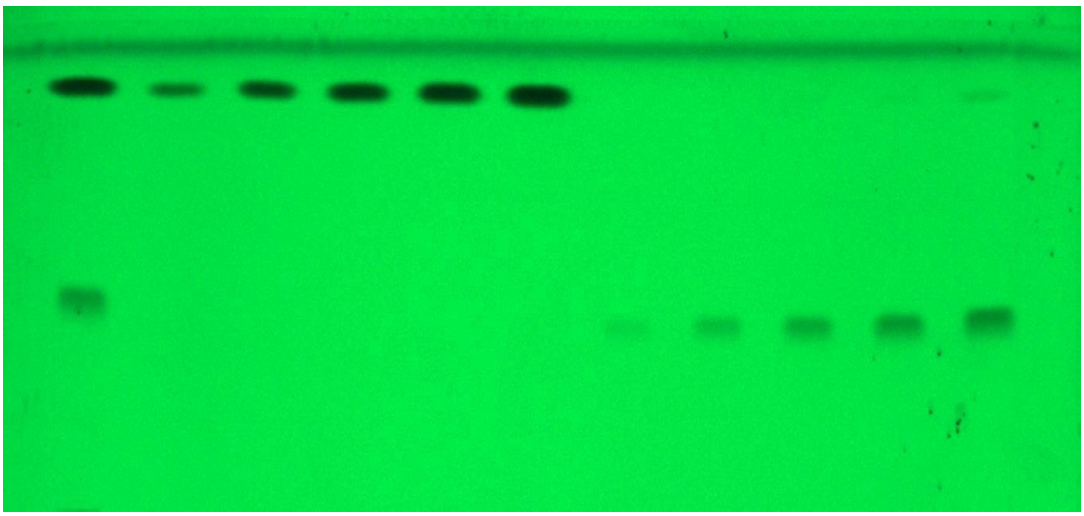


Figure 5: Photograph of HPTLC plate after development.

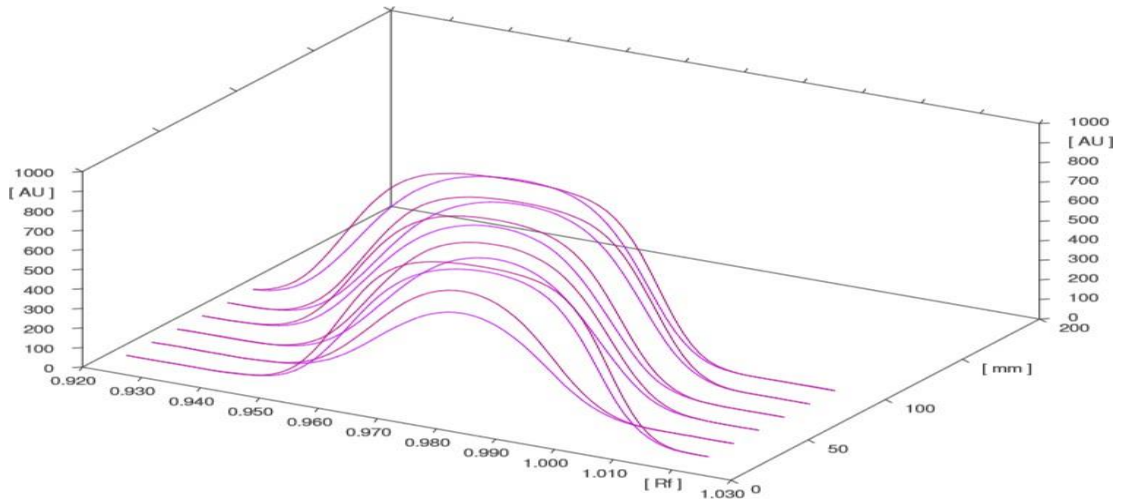


Figure 6: Spectra of the Cilnidipine and Chlorthalidone overlay.

Table 8: Chromatogram analysis data.

Track	Drug	Concentration (ng/spot)	Rf	Height	Area
1	CILNI	1000	0.98	424.4	9991.6
2	CILNI	3000	0.98	545.8	14376.6
3	CILNI	5000	0.97	646.2	18401.03
4	CILNI	7000	0.97	750.04	22012.29
5	CILNI	10000	0.97	880.13	26999.38
6	CHLOR	1000	0.75	100.25	2687.76
7	CHLOR	3000	0.76	185.3	6100.23
8	CHLOR	5000	0.77	251.14	9207.35
9	CHLOR	7000	0.77	313.63	12208.72
10	CHLOR	10000	0.76	395.87	16101.23
11.1	Standard drug mixture		0.98	747.43	27481.06
11.2			0.77	373.80	14951.90
12.1	Sample drug		0.98	745.60	27174.30
12.2			0.77	369.98	14943.18

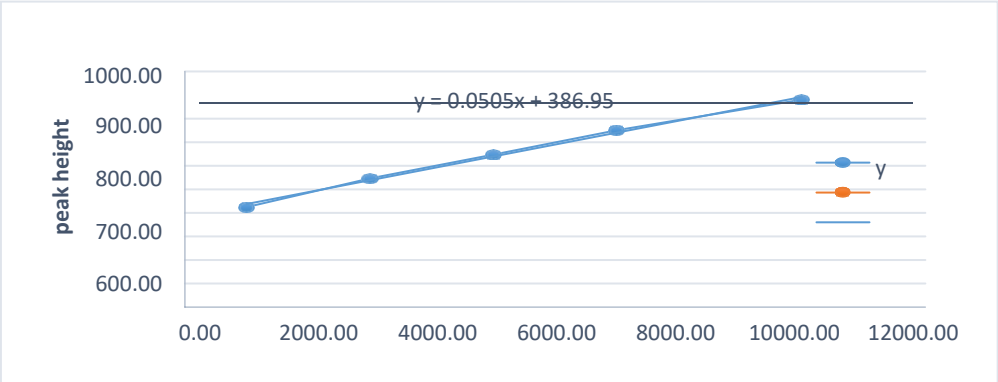


Figure 7: Calibration plot of cilnidipine (concentration v/s peak height).

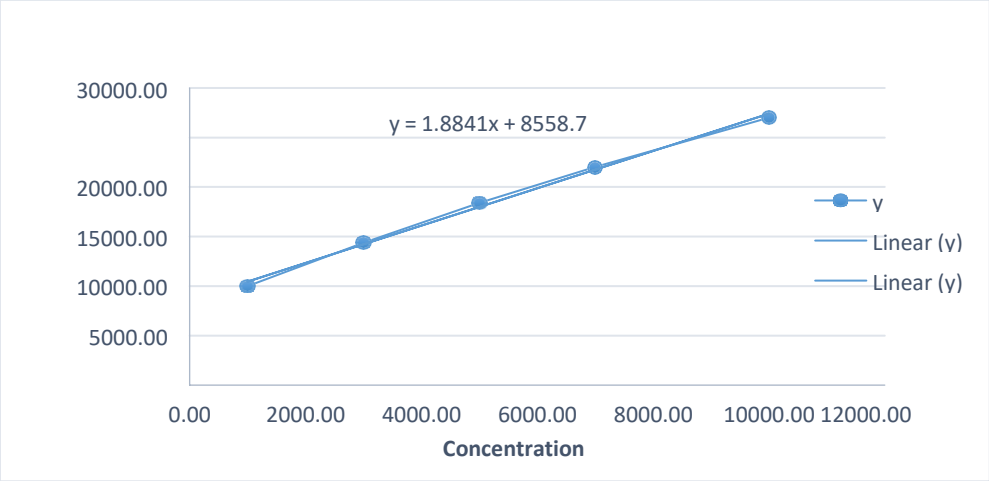


Figure 8: Calibration plot of cilnidipine (concentration v/s peak area).

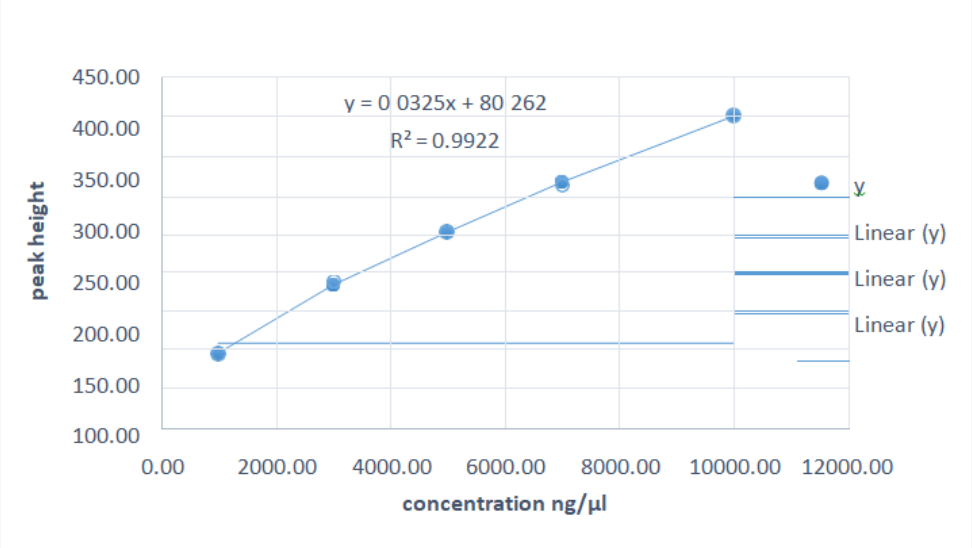


Figure 9: Calibration plot of chlorthalidone (concentration v/s peak height).

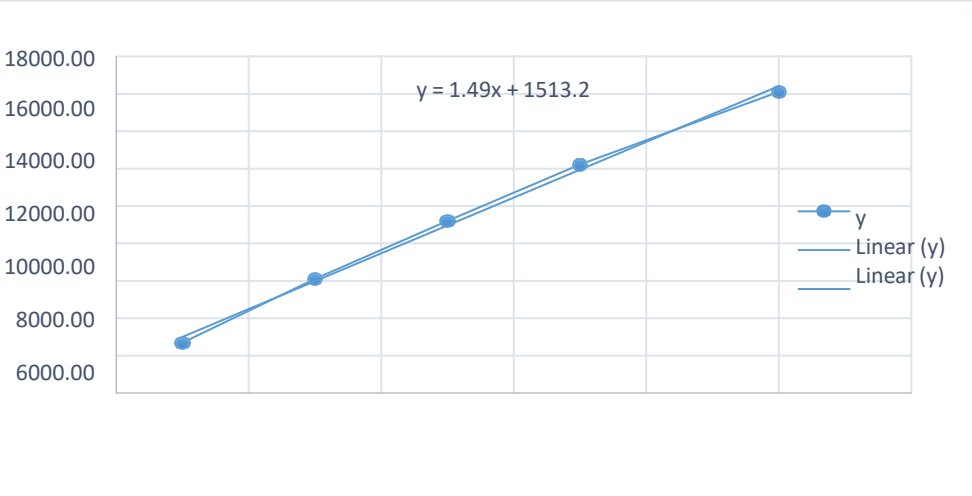


Figure 10: Calibration plot of chlorthalidone (concentration v/s peak area).

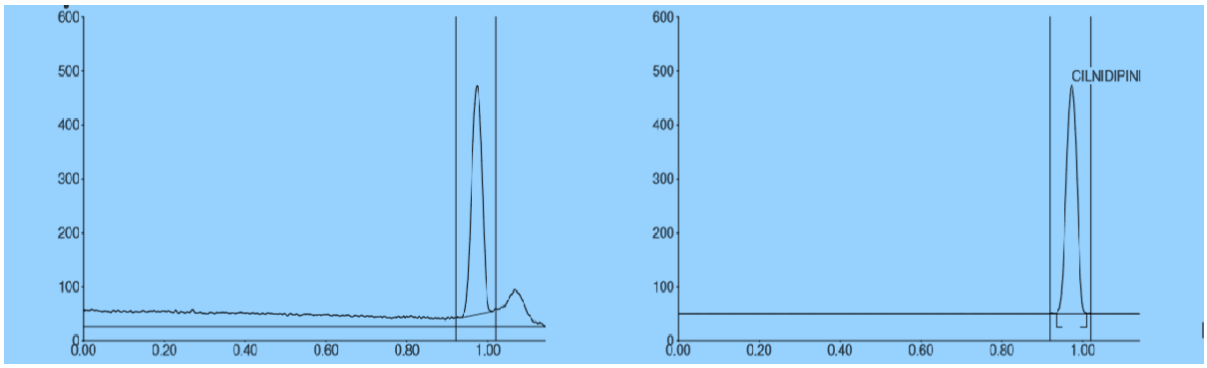


Figure 11: Cilnidipine hydrochloride 1000 ng.

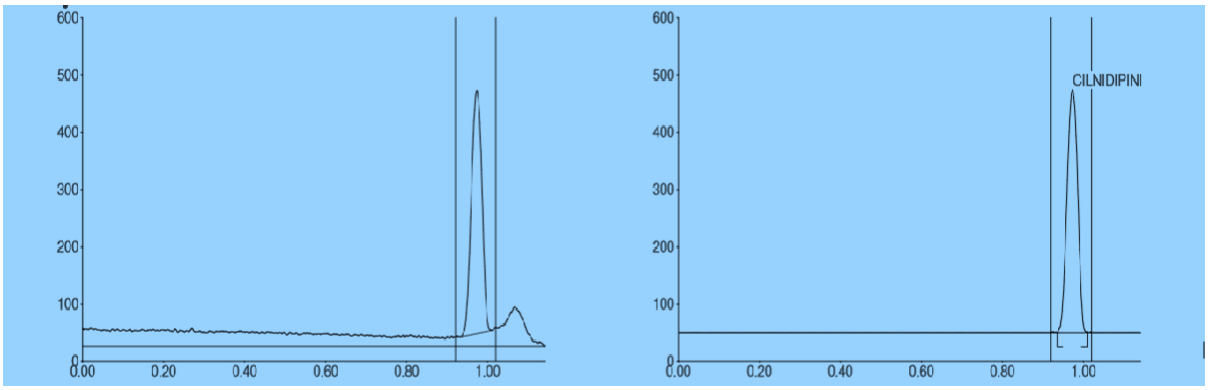


Figure 12: Cilnidipine hydrochloride 3000 ng.

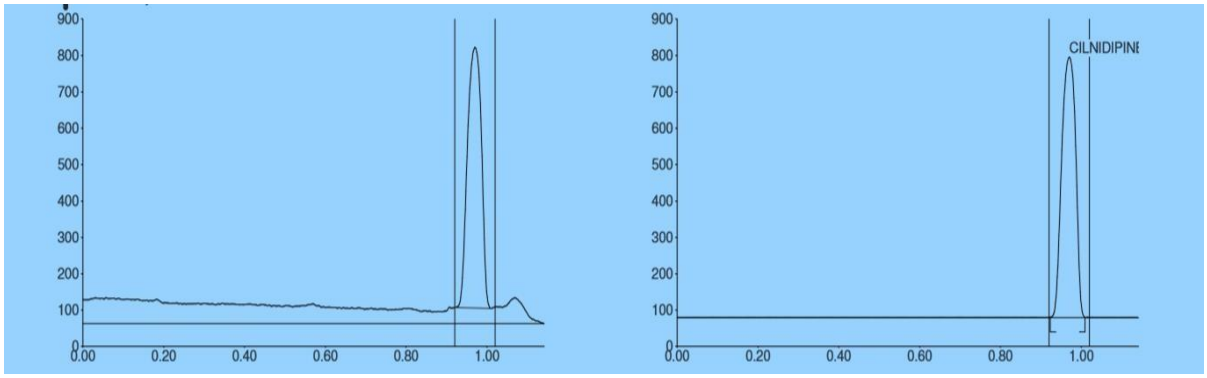


Figure 13: Cilnidipine hydrochloride 5000 ng.

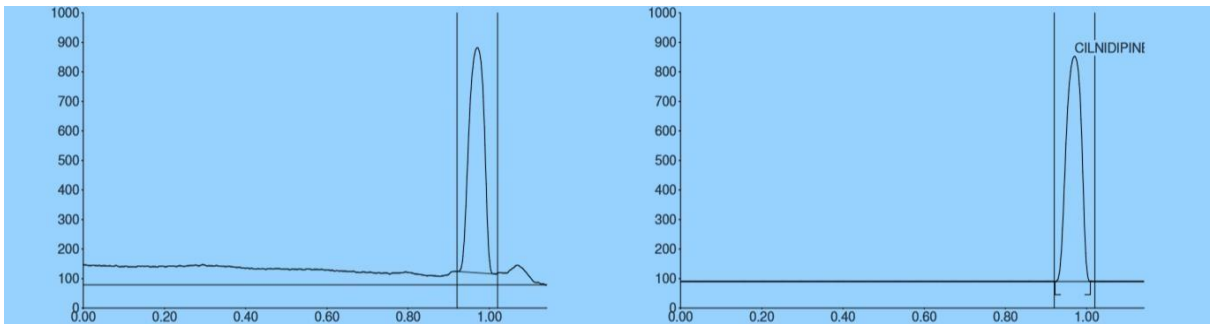


Figure 14: Cilnidipine hydrochloride 7000 ng.

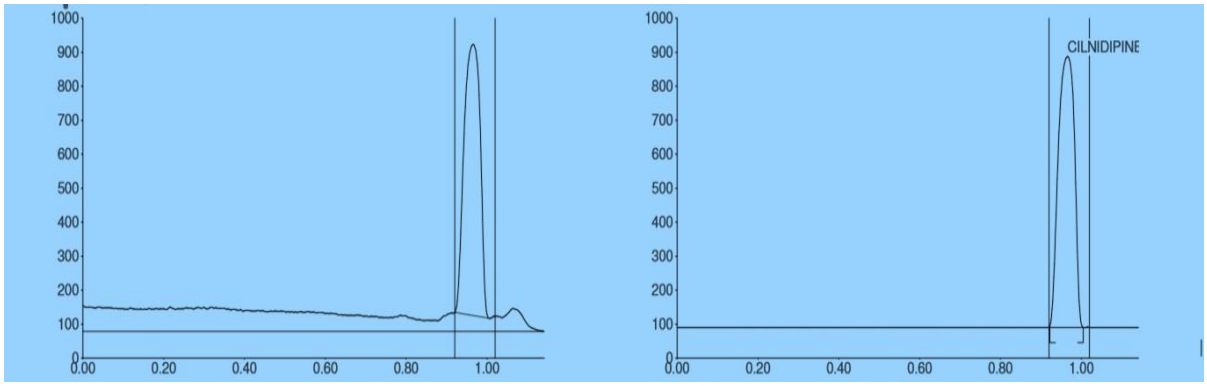


Figure 15: Cilnidipine hydrochloride 10,000 ng.

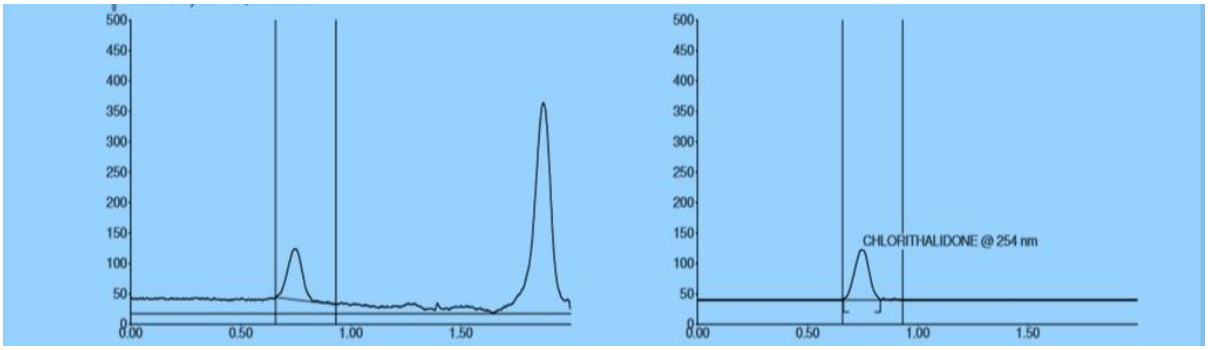


Figure 16: Chlorthalidone 1000 ng.

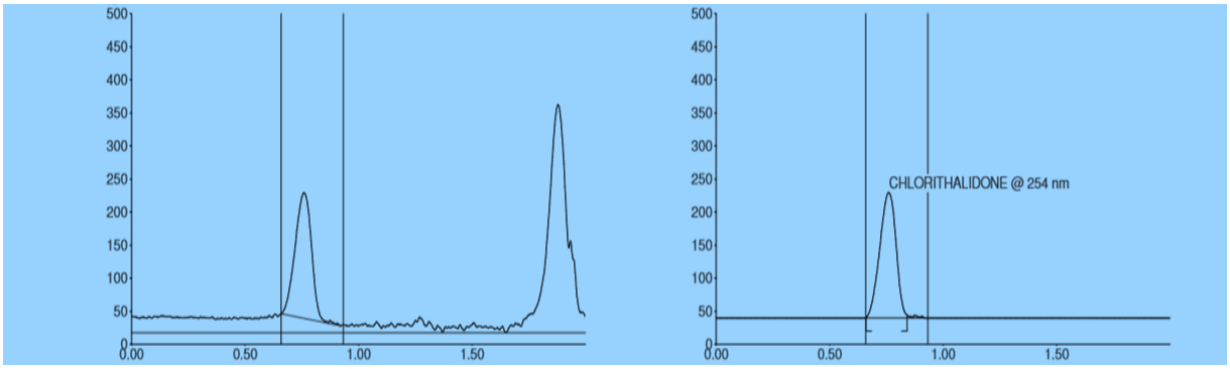


Figure 17: Chlorthalidone 3000 ng.

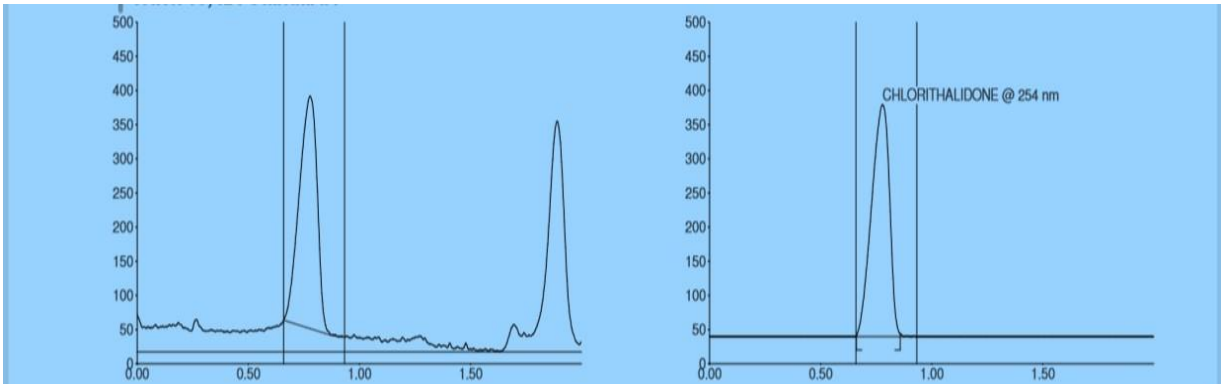


Figure 18: Chlorthalidone 7000 ng.

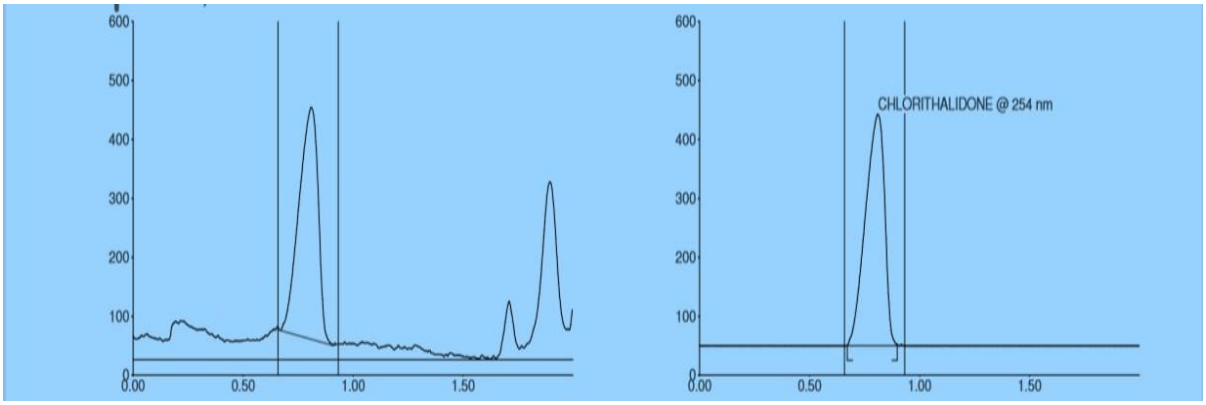


Figure 19: Chlorthalidone 10000 ng.

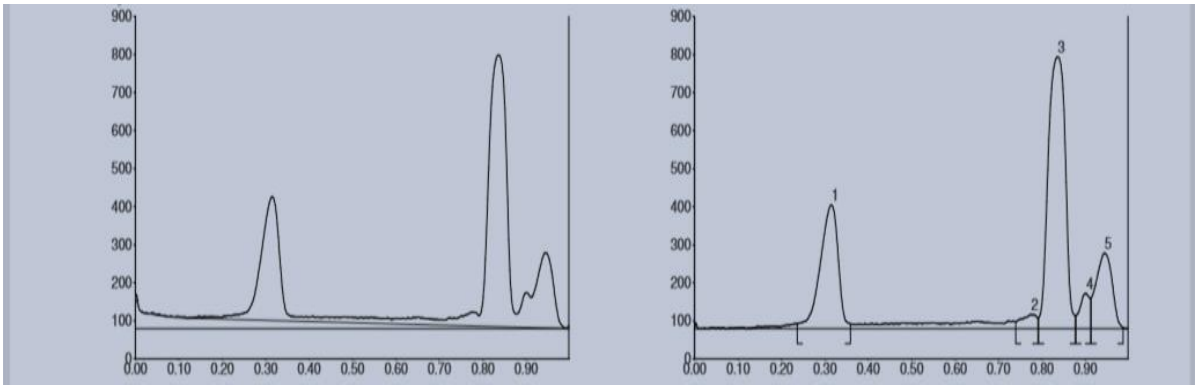


Figure 20: Standard drug mixture.

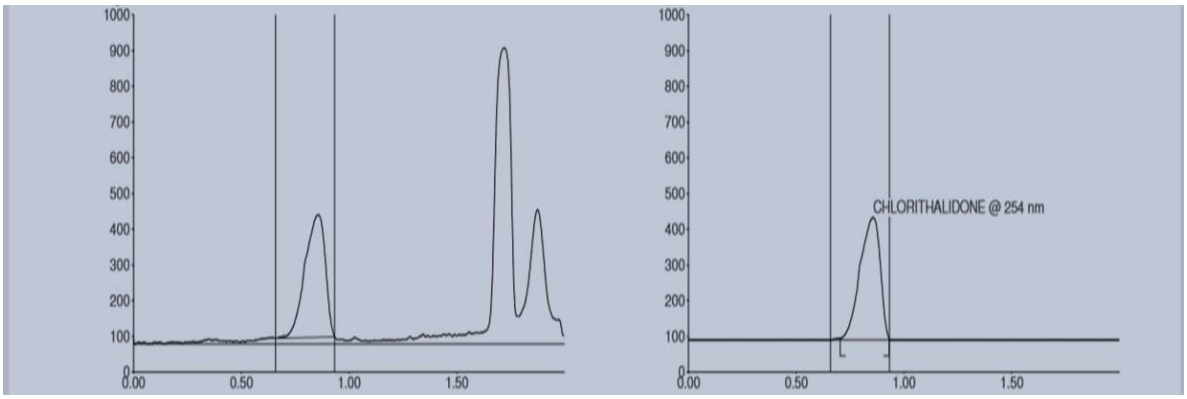


Figure 21: Sample drug solution – Chlorthalidone.

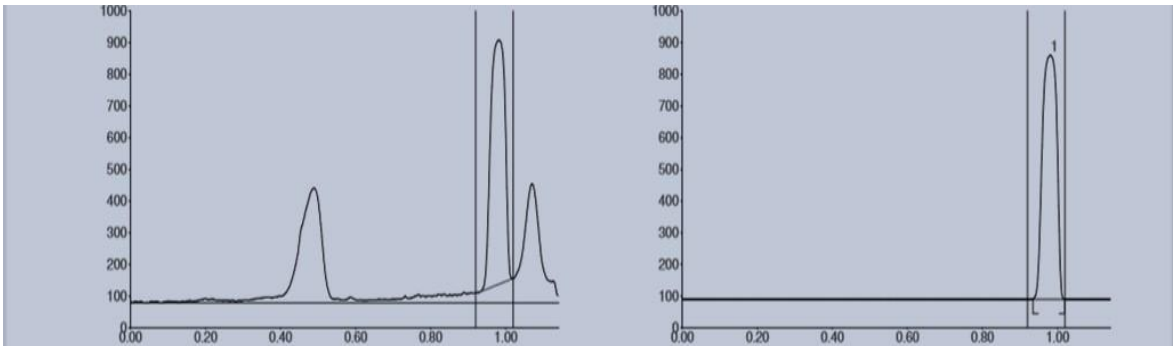


Figure 22: Sample solution- Cilnidipine hydrochloride.

RESULT

Each tablet contains (Label claim)

Cilnidipine hydrochloride : 10mg

Chlorthalidone : 6.25mg

Average weight of one tablet : 0.2615g

Weight equivalent 10 mg of Cilnidipine and 6.25 mg of Chlorthalidone: 0.2615gWeight taken : 0.2615

Table 9: Assay result of Cilacar- C (mg).

Drug	Drug content (mg)	
	Height wise	Area wise
Cilnidipine hydrochloride	9.97	9.83
Chlorthalidone	6.23	6.24

Table 10: Assay results of Cilacar- C.

Drug	Height wise (%w/w)	Area wise (%w/w)
Cilnidipine hydrochloride	99.75	98.83
Chlorthalidone	98.97	99.94

Validation of the proposed methodPrecision

Precision was done at two level- Intra-day precision and Inter-day precision

Intraday precision

Repeatability of the developed method was assessed by injectingstandard drug mixture of cilnidipine hydrochloride and chlorthalidone and the sample solution for six times and measured the area for all six injections in HPTLC on a single day.

Table 11: Intraday precision – Cilnidipine hydrochloride.

Sample ID	Peak height		Peak area	
	Standard	Sample	Standard	Sample
1	747.45	746.31	27481.08	26774.43
2	747.45	745.78	27481.08	26774.37
3	747.45	746.24	27481.08	26773.98
4	747.45	746.10	27481.08	26774.52
5	747.45	745.73	27481.08	26774.52
6	747.45	746.18	27481.08	26774.43

Table 12: Intraday precision – Chlorthalidone.

Sample ID	Area		Height	
	Standard	Sample	Standard	Sample
1	373.69	369.85	14952.01	14943.15
2	373.69	369.67	14952.01	14947.03
3	373.69	369.77	14952.01	14948.11
4	373.69	368.03	14952.01	14943.22
5	373.69	367.98	14952.01	14943.26
6	373.69	368.56	14952.01	14943.56

Intermediate/Inter – day precision

The inter – day precision study was carried out by scanning the chromatogram three times for three different concentrations.

Table 13: Results of inter – day precision – Cilnidipine hydrochloride.

SL. No	1st day		2nd day		3rd day	
	Height	Area	Height	Area	Height	Area
1	747.39	26778.12	746.71	26774.56	745.98	26778.57
2	747.42	26771.89	747.11	26778.01	747.45	26783.01
3	746.85	26780.09	745.97	26784.09	745.93	26775.87
4	747.15	26773.98	747.34	26779.45	746.77	26781.49
5	746.45	26774.93	747.27	26775.24	747.11	26783.28
6	747.37	26783.35	746.89	26781.09	747.38	26776.65

Table 14: Results of inter – day precision – Chlorthalidone.

Sample ID	Area		Height	
	Standard	Sample	Standard	Sample
1	373.69	369.85	14952.01	14943.15
2	373.69	369.67	14952.01	14947.03
3	373.69	369.77	14952.01	14948.11
4	373.69	368.03	14952.01	14943.22
5	373.69	367.98	14952.01	14943.26
6	373.69	368.56	14952.01	14943.56

Linearity and range

The linearity study was conducted to evaluate the linear relationship across the range of analytical procedure. Linearity was determined by using five different concentrations of each drug. Chromatogram was developed and peak area and peak height was determined by scanning at 235 nm. Calibration graphs (concentration/s peak height and concentration v/s peak area) were plotted for the drug and from this, the linearity was determined.

Table 15: Results of linearity data.

	Cilnidipine hydrochloride		Chlorthalidone	
	Height wise	Area wise	Height wise	Area wise
Linearity range	1-10 (µg/ml)	1-10	1-10(µg/ml)	1-10
Slope	0.0505	1.884	0.0325	1.49
Intercept	386.95	8558.7	80.262	1513.2
Correlation coefficient (r^2)	0.996	0.996	0.992	0.996

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were estimated from the set of 5 calibration curves used todetermine the linearity of the developed method. Five calibration curves were drawn for the drugs that come across within its linearity range. From each calibration curve y-intercept and slope were determined and are substituted in the corresponding equation for finding the LOD and LOQ.

$$LOD\ 3.3\ \sigma/SLOQ = 10\ \sigma/S$$

Where,

σ=Standard deviation of y-intercepts

S= slope of the calibration curves

Table 16: LOD and LOQ data.

Drug	Method	Slope	Standard deviation	LOD ng/spot	LOQ ng/spot
Cilnidipine Hydrochloride	Height wise	0.0505	0.0007	0.0457	0.1386
	Area wise	1.884	0.1643	0.2877	0.8720
Chlorthalidone	Height wise	0.0325	0.0066	0.6701	2.0307
	Area wise	0.0443	0.0127	0.9460	2.8668

RESULTS AND DISCUSSION

All the procedures were done in CAMAG HPTLC instrument. Methanol was used for the preparation of sample and standard solutions. HPTLC pre coated silica gel G60 F 254 plates were used as stationary phase. By trial-and-error process, a suitable mobile phase was developed –Ethyl acetate: Hexane (6:4 v/v). All the solvents used were HPLC grade. Spotting was done by using CAMAG LINOMAT IV automatic sample applicator with a band width of 4mm and an application volume of 10µl. The developed plate was scanned at 235 nm, The Rf value of cilnidipine hydrochloride was 0.98 and that of chlorthalidone was 0.77. Calibration curve for each drug was plotted using two parameters –peak height and peak area. The linearity range of cilnidipine hydrochloride and chlorthalidone was found to be 1000 -10,000 ng/spot. Cilnidipine, the marketed product was analyzed by the developed method and gave good results. The label claim for cilnidipine hydrochloride was 99.97 % v/v (height wise) and 10.14 % v/v (area wise) and for chlorthalidone 99.52 % v/v (height wise) and 99.94 % v/v (area wise). The validation of the developed method was conducted as per ICH guidelines. The precision was studied by two methods –Intraday precision and inter-day precision. The percentage RSD was found to be less than 2% both area wise and height wise. The LOD and LOQ were determined and satisfactory results obtained. The developed method was found to be simple, economic and accurate. So, it can be used for routine analysis of the particular combination.

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