An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

NEW VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANANEOUS ESTIMATION OF CLINIDIPINE AND CHLORTHALIDONE IN HUMAN PLASMA

G. Dharmamoorthy*[1], K.S.Nataraj[2], A. Krishna Manjari.Pawar[3]

[1] Research scholar, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam- 53003, Andhra Pradesh, India

[2]Department of Pharmaceutical Analysis, Shri Vishnu college of Pharmacy, Bhimavaram.-534201 Andhra Pradesh. India

[3] Department of pharmaceutical Analysis, University College of Pharmaceutical sciences .Andhra University, Visakhapatnam.53003, Andhra Pradesh, India

*Corresponding author E-mail: dharmamoorthy111@gmail.com

ARTICLE INFO

Key Words

ICH guidelines, Chlorthalidone, Clindapine, K_2EDTA, Plasma, Celecoxib Internal standard , RP-HPLC, Validation

Access this article online

Website: https://www.jgtps.com/

Quick Response Code:

ABSTRACT

Objective;A new simple and precise stability indicating bioanalytical reverse-phase High Performance Liquid Chromatography(RP-HPLC) method was developedand validated for the simultaneous estimation of Clindipine and Chlorthalidone in their formulation and in human plasma. **Materials and methods:** The developed method was successfully used for assaying drug contents in plasma. Isocratic elution mode was carried at Phenomenex C18 (150mm x 4.6 mm, 5µ)using 0.01N disodium hydrogenphosphate pH 5.0; Acetonitrile (60;40) as mobile phase at flow rate 1.0ml/min at detection wavelength 220 nm .Azilsartan was taken as an internal standandard. **Results:** The method was validated as per ICH guidelines. It is concluded that the present validated method can be successfully applied for the estimation of Clindipine and Chlorthalidonein in human plasma over the concentration range of 0.03 to 1.2 µg /ml of Chlorthalidoone, 0.015 to 0.6 µg /ml of Clindapine. The method for determination of Clindipine and Chlorthalidone in human plasma using HPLC detection met the acceptance criteria with respect to selectivity, precision, accuracy, linearity, recovery. **Conclusion; ;** The proposed method is simple, rapid, accurate, precise, and appropriate for pharmacokinetic and therapeutic drug monitoring in the clinical laboratories.

INTRODUCTION

Chlorthalidone is an antihypertensive/diuretic .[1,2] ·It is a monosulfamyl diuretic that differs chemically from thiazide diuretics in that a double ring system is incorporated in its structure. It is a racemic mixture of 2-chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl) benzene sulfonamide. Chlorthalidone prevents reabsorption of sodium and chloride through inhibition of the Na+/Cl- symporter in the Cortical diluting segment of the ascending limb of the loop of Henle.4 Reduction of sodium reabsorption subsequently reduces extracellular fluid and plasma volume via an osmotic, sodium-driven diuresis.

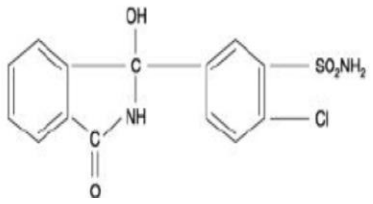

Fig 1: Chemical Structure of Chlorthalidone

Cilnidipine is a dihydropyridine calcium antagonist [3,4]. It was jointly developed by Fuji Viscera Pharmaceutical Company, Japan and Ajinomoto, Japan and approved in 1995. Compared with other calcium antagonists, cilnidipine can act on the N-type calcium channel that existing sympathetic nerve end besides acting on L-type calcium channel that similar to most of the calcium antagonists.Cilnidipine acts on the L-type calcium channels of blood vessels by blocking the incoming calcium and suppressing the contraction of blood vessels, thereby reducing blood pressure. Cilnidipine also works on the N-type calcium channel located at the end of the sympathetic nerve, inhibiting the emission of norepinephrine and suppressing the increase in stress blood pressure.

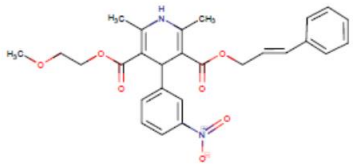

Fig 2: Chemical Structure of Clindapine

Literature survey reveals that few methods have been reported for the simultaneous estimation of Chlorthalidone and Clindapine by using spectroscopic and chromatographic methods [5-12]. But there is no reported for the bioanalytical methods. The main aim of the present study is to develop a precise, sensitive, accurate, selective, reproducible and rapid analytical technique for estimation of Chlorthalidone and Clindapine in human plasma and validate as per ICH guidelines. [13-17]

Materials and Methods

Materials:

API: Chlorthalidone and Cilnidipine API was obtained as a gift sample from Spectrum pharma research solutions.

Human plasma: K2 EDTA control plasma procured by Deccan Pathological labs, Hyderabad

Method Development

Diluent: Based up on the solubility of the drugs, diluent was selected, 0.01NPotassium dihydrogen phosphate and Acetonitrile taken in the ratio of 50:50.

Preparation of Chlorthalidone Stock solution (60 µg/ml): Take 6 mg of Chlorthalidone in 100 ml volumetric flask and make the volume with diluent to produce 60µg/ml.

Preparation of Chlorthalidone Spiking Solutions: From the above Chlorthalidone stock solution 0.05ml, 0.1ml, 0.15ml, 0.6ml, 1.0ml, 1.2ml, 1.6ml and 2.0 ml was pipette and transferred to 8 individual 10 ml volumetric flask and make up the volume up to the mark with diluent to produce 0. 3µg/ml, 0.6 µg/ml, 0.9 µg/ml, 2.4 µg/ml, 6.0 µg/ml, 7.2µg/ml, 9.6 µg/ml and 12µg/ml.Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 0.03µg/ml, 0.06 µg/ml, 0.09 µg/ml, 0.24 µg/ml, 0.60 µg/ml, 0.72µg/ml, 0.96 µg/ml and 1.2µg/ml.

Preparation of Cilnidipine Stock solution (30 µg/ml): Take 3 mg of Cilnidipine in 100 ml volumetric flask and make the volume with diluent to produce 30 µg/ml.

Preparation of Cilnidipine Spiking Solutions: From the above Cilnidipine stock solution 0.05ml, 0.1ml, 0.15ml, 0.6ml, 1.0ml, 1.2ml, 1.6ml and 2.0 ml was pipette and transferred to 8 individual 10 ml volumetric flask and make up the volume up to the mark with diluent to produce 0.15 µg/ml, 0.30µg/ml, 0.45µg/ml, 1.2 µg/ml, 3.0 µg/ml, 3.6 µg/ml, 4.8 µg/ml and 6.0 µg/ml. Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 0.015 µg/ml, 0.03µg/ml, 0.045µg/ml, 0.12 µg/ml, 0.3 µg/ml, 0.36 µg/ml, 0.48 µg/ml and 0.6 µg/ml.

Preparation of internal standard Solution (Azilsartan): Stock-1: Take 50 mg of Azilsartan in 100 ml volumetric flask and make up the volume with diluent to produce 500µg/ml.

Stock-2: From the above solution, take 1ml of solution into 10 ml volumetric flask and make up the volume with diluent to produce 50µg/ml solutions.Final concentration: From the above solution, take 0.5ml of solution and spiking blank plasma with workingstock dilutions of analyte to produce 10µg/ml ISD concentration

Extraction procedure: Take 750µl of plasma and 500µl of internal standard, 250µl of Chlorthalidone from the spiking solutions of both into a centrifuging tube and add 1 ml of Acetonitrile go for cyclomixer for 15 sec. Then

vertex for 2 min and finally centrifuge for 5 min at 3200 rpm speed. After the centrifugation collect the sample and filter it directly inject 50 µL into HPLC.

Optimized Chromatographic conditions

Mobile phase: 0.01N Potassium dihydrogen phosphate pH (3.0): Acetonitrile (60:40)

Flow rate: 1.0ml/min, Column: Phenomenex c18 C18 (150mm x 4.6 mm, 5µ), Detector wavelength : 220nm, Column temperature: 30^0C, Injection volume : 50µL

Method Validation

System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines. The % CV for system suitability test was in the range of 0.26 for Retention time (RT) of Chlorthalidone , 0.94 for Retention time (RT) of cilnidipine and 0.38% for the area ratio (analyte area/IS area) of Azilsartan.

Auto sampler Carryover test: Due to the auto-sampler was investigated by injecting a sequence of un-extracted and extracted samples. Results demonstrated that no significant carry over was observed during this experiment.

Matrix factor evaluation: Matrix effect is played a vital role in the assessment of pharmacokinetic studies. It was expressed as internal standard normalized matrix factor and it was varied from 0.90-0.99 which was close to 1 which indicates there is no ionization suppression or enhancement in plasma samples.

Quality control samples: The chromatography observed during the course of Chlorthalidone and Cilnidipine was acceptable and representative chromatograms of standard Blank, standard zero (standard blank with internal standard) QC-LLOQ, QC-L, QC-M1, QC-M2 and QC-H samples.

Selectivity/Specificity: To establish the selectivity of the method, possible interference at the retention time of Chlorthalidone& Cilnidipineand Internal standard due to endogenous plasma components were checked during the validation. Selectivity was performed by testing six batches of K_2EDTA blank plasma and the mass detection of extracted blank plasma gave good selectivity of both drug and internal standard. No interferences were found at the retention times of analyses and internal standard. Representative chromatograms of standard blank and blank with internal standard sample using pooled plasma.

Linearity: Calibration was found to be linear over the concentration range of 0.030 to 1.2 µg /ml for Chlorthalidone, 0.015 to 0.6 µg /ml Cilnidipine. The coefficient correlation (r^2) value was found consistently greater than 0.999 in all the cases. This indicating linearity of results and an excellent correlation between peak area ratios for each concentration of analytes. A representative calibration curve is obtained during the third precision and accuracy batch.

Precision and Accuracy: The intraday and inter day accuracy and precision was assessed by analysing six replicates at five different QC levels like LLOQ, LQC, MQC and HQC. Accuracy and precision method performance was evaluated by determined by six replicate analyses for Chlorthalidone at four concentration levels, i.e.,0.03µg/ml(LLOQ), 0.090 µg/ml (LQC), 0.60 µg/ml (MQC) and 0.96 µg/ml (HQC), Cilnidipine at 0.015µg/ml(LLOQ), 0.045 µg/ml (LQC), 0.3 µg/ml (MQC) and 0.48 µg/ml (HQC),The intra-day and inter day accuracy of plasma samples were assessed and excellent mean % accuracy was obtained with range varied from 99.96-100.35%, and 98.99%-99.93 % for intraday and 99.06%-100.02 and 98.91%-100.24 for inter day respectively. The precision (%CV) of the analytes and plasma samples were calculated and found to be 0.38-11.54% and 0.76%-13.49% for intraday and 0.66%-14.23% and 0.77 %-13.16% for inter day respectively.

Recovery: Recovery was determined by measuring the peak areas obtained from prepared plasma samples with those extracted blank plasma spiked with standards containing the same area with known amount of Chlorthalidone and Cilnidipine. The overall % mean recovery for Chlorthalidone and Cilnidipine was found to be 98.12% and 98.06%. The recoveries obtained for Chlorthalidone and Cilnidipine at 3 QC concentration levels are summarized in the results and discussion. The overall % mean recovery for Azilsartan was found to be 98.11%.

Table 1: List of chemicals and Solvents

S.no	Chemical name	Grade	Manufacturing company
1	Distilled water HPLC water	HPLC	Rankem, Avantor performance material India limited
2	HPLC water	Analytical Reagent	Rankem, Avantor performance material India limited
3	Acetonitrile	Analytical Reagent	Rankem, Avantor performance material India limited
4	Phosphate buffer	Analytical Reagent	Rankem, Avantor performance material India limited
5	Methanol	Analytical Reagent	Rankem, Avantor performance material India limited
6	Sodium dihydrogen phosphate	Analytical Reagent	Rankem, Avantor performance material India limited
7	Ortho-phosphoric acid	Analytical Reagent	Rankem, Avantor performance material India limited

Table 2: List of Instruments

S.no	Instrument	Company name	Brand name
1	Electronic balance	Sartorious	Denver
2	pH meter	Metsar	BVK enterprises
3	Sonicator	Lab man	BVK enterprises
4	Centrifuge	Thermo Fisher	-
5	Vertex	Remi CM101	-
6	HPLC water	Alliance	Water HPLC 2695 SYSTEM

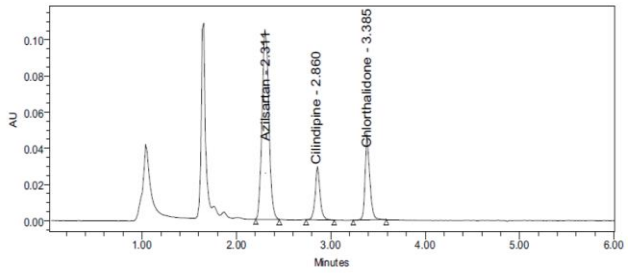

Fig 3: Optimized Chromatogram

Results and Discussion

Table 3: System suitability of Chlorthalidone

System Suitability						
Validation No.		SOP No.			Column ID.	
Analyte	Chlorthalidone	ISTD	Azilsartan			
Acquisition Batch ID					DATE	
Sample Name	File Name	Analyte Area	Analyte RT (min)	ISTD Area	ISTD RT (min)	Area Ratio
AQ MQC		27550	3.39	65259	2.31	0.4222
AQ MQC		27685	3.38	65342	2.33	0.4237
AQ MQC		27989	3.36	65589	2.35	0.4267
AQ MQC		27785	3.38	65438	2.34	0.4246
AQ MQC		27694	3.37	65359	2.32	0.4237
AQ MQC		27596	3.38	65287	2.33	0.4227
MEAN			3.379		2.329	0.42393
SD			0.0087		0.0152	0.001617
%CV			0.26		0.65	0.38

Table 4: System suitability of Cilnidipine

System Suitability						
Validation No.		SOP No.			Column ID.	
Analyte	Cilnidipine	ISTD	Azilsartan			
Acquisition Batch ID					DATE	
Sample Name	File Name	Analyte Area	Analyte RT (min)	ISTD Area	ISTD RT (min)	Area Ratio
AQ MQC		14765	2.86	65259	2.31	0.2263
AQ MQC		14985	2.83	65342	2.33	0.2293
AQ MQC		15034	2.79	65589	2.35	0.2292
AQ MQC		14987	2.86	65438	2.34	0.2290
AQ MQC		15146	2.84	65359	2.32	0.2317
AQ MQC		14698	2.85	65287	2.33	0.2251
MEAN			2.839		2.329	0.22845
SD			0.0268		0.0152	0.002381
%CV			0.94		0.65	1.04

Table 5: Auto sampler carryover of Chlorthalidone

Acquisition Batch ID			Date	
Sample ID	Peak Area		% Carryover	
	Drug	ISTD	Drug	ISTD
Unextracted samples				
RS	0	0	N/A	N/A
AQ ULOQ	56486	66756	0.00	0.00
RS	0	0		
AQ LLOQ	1526	67128	N/A	N/A
Extracted samples				
STD Blk	0	0	N/A	N/A
ULOQ	55099	65457	0.00	0.00
STD Blk	0	0		
LLOQ	1380	65249	N/A	N/A

Table 6: Auto sampler carryover of Cilnidipine

Acquisition Batch ID			Date	
Sample ID	Peak Area		% Carryover	
	Drug	ISTD	Drug	ISTD
Unextracted samples				
RS	0	0	N/A	N/A
AQ ULOQ	31680	66756	0.00	0.00
RS	0	0		
AQ LLOQ	864	67128	N/A	N/A
Extracted samples				
STD Blk	0	0	N/A	N/A
ULOQ	29536	65457	0.00	0.00
STD Blk	0	0		
LLOQ	740	65249	N/A	N/A

Table 7: Matrix factor evaluation of Chlorthalidone (absence of matrix factor)

Acquisition Batch ID		Date	
S. No.	Plasma Lot No.	HQC	LQC
		Nominal Concentration (ng/mL)	
		960.000	90.000
		Nominal Concentration Range (ng/mL)	
		(816.000-1,104.000)	(76.500-103.500)
		Calculated Concentration (ng/mL)	
1	LOT1	953.97	86.15
		954.00	84.15
		951.94	81.14
2	LOT2	953.91	83.14
		958.03	85.14
		956.88	88.13
3	LOT3	963.06	82.13
		969.83	90.15
		970.09	94.16
4	LOT4	978.81	91.15
		972.12	93.16
		979.77	98.16
5	LOT5	974.09	96.15
		971.74	92.17
		985.81	102.17
6	LOT6	981.78	100.16
		987.15	99.17
		984.01	103.16
n		18	18
Mean		969.2778	91.6528
SD		12.19584	7.11650
% CV		1.26	7.76
% Mean Accuracy		100.97	101.84
No. of QC Failed		0	0

Quality control samples
Standard zero sample:

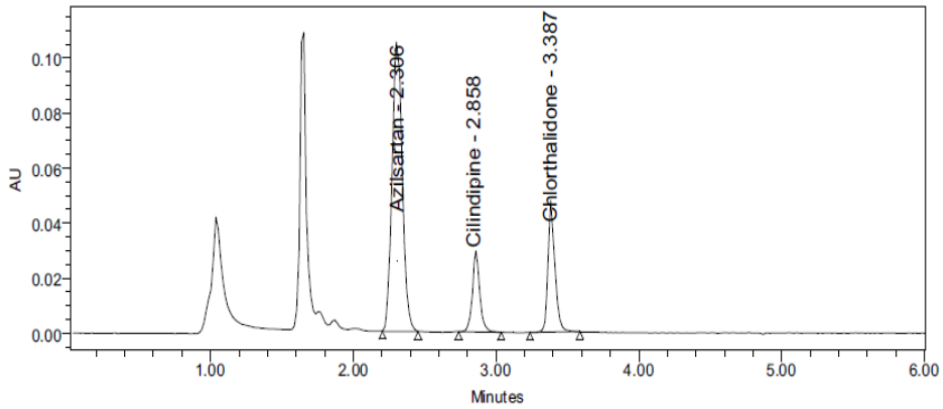

Fig 4: Chromatogram of standard Zero sample QC-LLOQ

Table 8: Matrix factor evalution of Cilnidipine

Acquisition Batch ID		Date	
S. No.	Plasma Lot No.	HQC	LQC
		Nominal Concentration (ng/mL)	
		480.000	45.000
		(408.000-552.000)	(38.250-51.750)
		Calculated Concentration (ng/mL)	
1	LOT1	486.980	41.073
		481.979	40.076
		482.041	44.074
2	LOT2	478.033	48.076
		476.011	37.074
		477.931	39.072
3	LOT3	491.034	46.071
		492.940	43.075
		493.976	42.075
4	LOT4	489.090	41.071
		485.928	45.072
		501.018	42.071
5	LOT5	491.957	41.072
		502.126	47.071
		490.058	46.076
6	LOT6	492.872	50.075
		504.941	51.076
		493.086	59.076
n		18	18
Mean		489.5556	44.6292
SD		8.31022	5.22768
% CV		1.70	11.71
% Mean Accuracy		101.99	99.18
No. of QC Failed		0	2

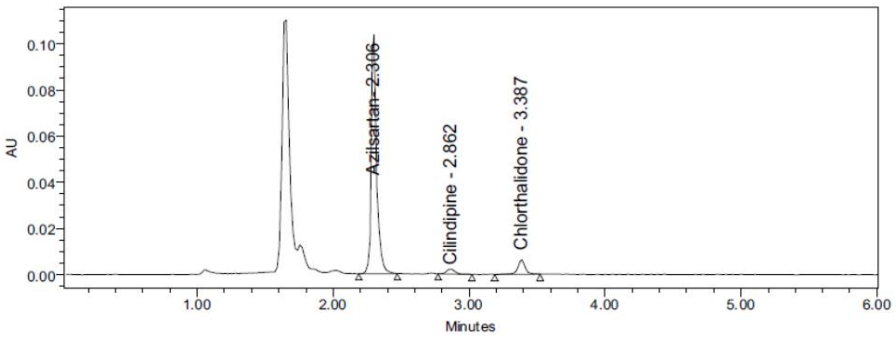

Fig 5: Chromatogram of QC-LLOQ sample Cilindipine, Chlorthalidone

QC-LQC

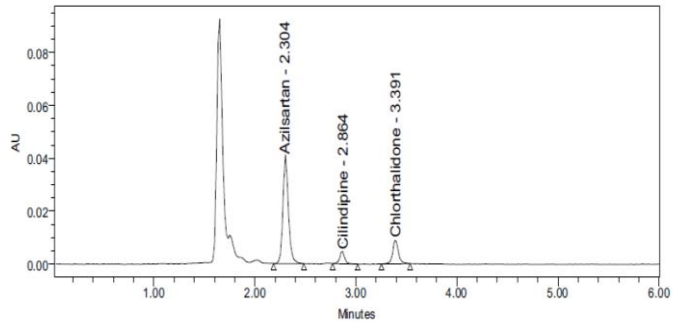

Fig 6: Chromatogram of QC-LQC sample Chlorthalidone, Cilnidipine and

QC-MQC

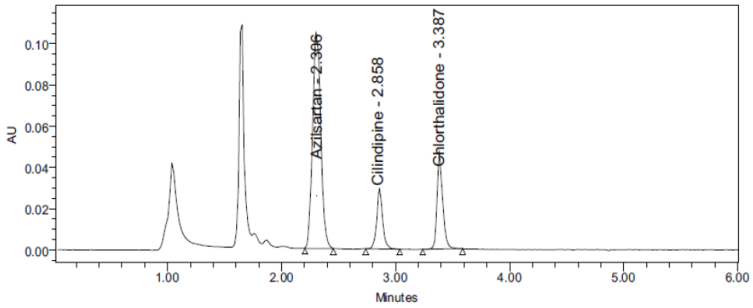

Fig 7: Chromatogram of QC-MQC sample Chlorthalidone, Cilnidipine and QC-HQC

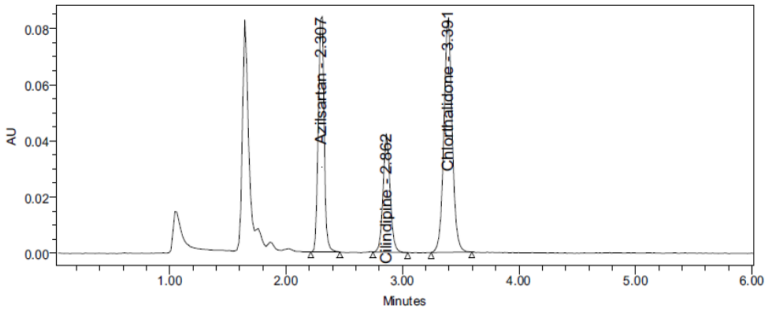

Fig 8: Chromatogram of QC-HQC sample Chlorthalidone, Cilnidipine

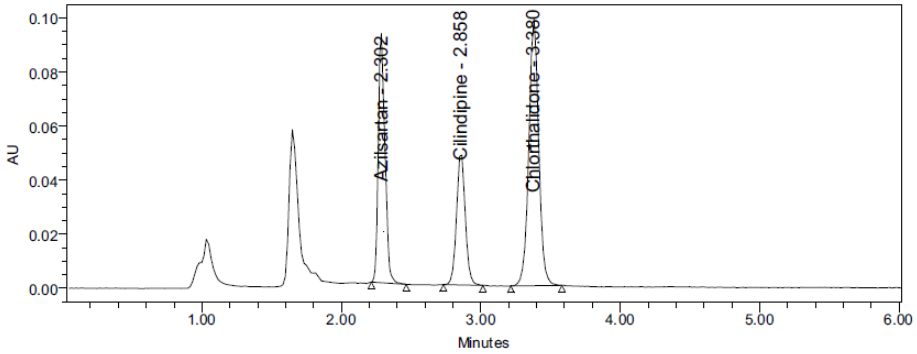

Fig 9: Chromatogram of ULOQ sample Chlorthalidone& Cilnidipine

Table 9:Linearity of Chlorthalidone

S.no	Final conc. in µg/m	ISD(area)	Drug(area)	Area ratio
1	0.03	65259	1387	0.021
2	0.06	65485	2768	0.042
3	0.09	65692	4157	0.063
4	0.24	65543	11086	0.169
5	0.6	65756	27698	0.421
6	0.72	65847	33184	0.504
7	0.96	65478	44187	0.675
8	1.2	65379	54281	0.830

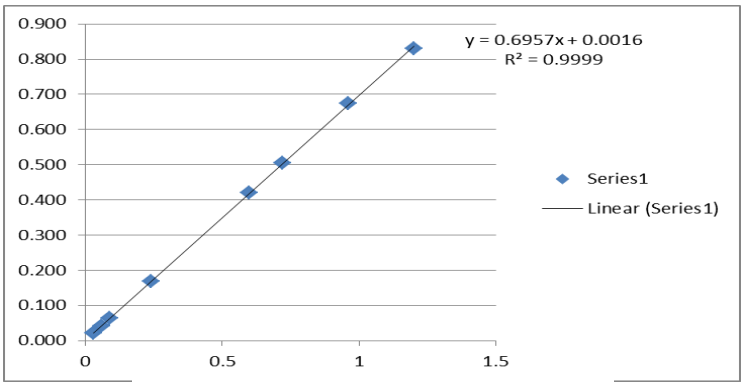

Fig 10:Calibration curve of Chlorthalidone

Table 10: Linearity of Cilnidipine

S.no	Final conc. in µg/m	ISD(area)	Drug(area)	Area ratio
1	0.015	65259	740	0.0113
2	0.03	65485	1468	0.0224
3	0.045	65692	2289	0.0348
4	0.12	65543	5965	0.0910
5	0.3	65756	14857	0.2259
6	0.36	65847	17798	0.2703
7	0.48	65478	23692	0.3618
8	0.6	65379	28633	0.4380

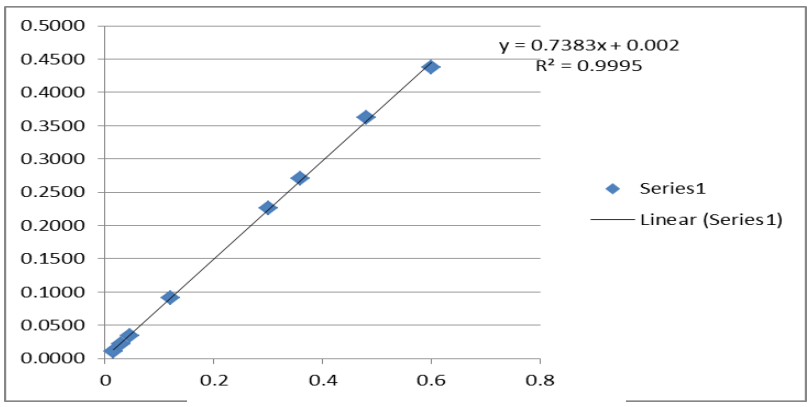

Fig 11:Calibration curve of Cilnidipine

Table 11:Precision&Accuracy (intra-day runs of Chlorthalidone)

P&A ID	Acquisition Batch ID	HQC	MQC1	LQC	LLOQ QC
		Nominal Concentration (ng/mL)			
		960.000	600.000	90.000	30.000
		Nominal Concentration Range (ng/mL)			
		(816.000-1,104.000)	(510.000-690.000)	(76.500-103.500)	(24.000-36.000)
		Calculated Concentration (ng/mL)			
Different Column		953.997	600.997	87.147	27.048
		957.000	603.000	91.150	31.051
		954.994	592.994	88.144	34.045
		963.300	593.300	94.153	28.054
		960.991	597.991	95.147	35.051
		965.600	605.400	92.150	25.057
N		6	6	6	6
Mean		959.3137	598.9470	91.3152	30.0510
SD		4.70110	5.10904	3.19031	3.99745
% CV		0.49	0.85	3.49	13.30
% Mean Accuracy		99.93	99.82	101.46	100.17
Different Analyst		957.998	597.997	87.148	30.047
		952.200	592.000	92.151	27.051
		954.995	594.993	94.145	24.044
		965.500	604.300	85.154	28.054
		961.200	601.990	91.142	35.051
		968.990	607.200	84.157	34.057
N		6	6	6	6
Mean		960.1472	599.7467	88.9828	29.7173
SD		6.36047	5.77905	4.06602	4.22971
% CV		0.66	0.96	4.57	14.23
% Mean Accuracy		100.02	99.96	98.87	99.06

Table 12:Precision&Accuracy (intra-day runs of Cilindipine)

P&A ID	Acquisition Batch ID	HQC	MQC1	LQC	LLOQ QC
		Nominal Concentration (ng/mL)			
		480.000	300.000	45.000	15.000
		Nominal Concentration Range (ng/mL)			
		(408.000-552.000)	(255.000-345.000)	(38.250-51.750)	(12.000-18.000)
		Calculated Concentration (ng/mL)			
Different Column		478.997	302.245	46.074	15.023
		481.964	305.215	49.071	18.000
		480.000	303.275	48.077	12.025
		475.931	298.242	43.068	13.022

		482.032	295.305	42.079	16.028
		473.928	293.212	45.066	17.025
N		6	6	6	6
Mean		478.8087	299.5823	45.5725	15.1872
SD		3.28337	4.75659	2.73851	2.31131
% CV		0.69	1.59	6.01	15.22
% Mean Accuracy		99.75	99.86	101.27	101.25
Different Analyst		483.998	306.247	41.072	18.000
		480.968	300.250	46.075	13.024
		486.007	303.243	48.069	16.021
		481.937	298.253	49.078	14.027
		478.030	295.240	39.066	12.923
		475.933	292.256	45.081	15.027
N		6	6	6	6
Mean		481.1455	299.2482	44.7402	14.8370
SD		3.72305	5.13599	3.93567	1.95187
% CV		0.77	1.72	8.80	13.16
% Mean Accuracy		100.24	99.75	99.42	98.91

Table 13: Recovery of Chlorthalidone

Acquisition Batch ID						
Replicate No.	HQC		MQC1		LQC	
	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response
1	45156	44080	28145	27650	4245	4165
2	45031	44259	28278	27885	4712	4678
3	44977	44387	28465	27988	4578	4456
4	45179	44230	28573	28052	4638	4523
5	45046	44237	28355	27746	4456	4358
6	44985	44198	28245	27659	4662	4569
n	6	6	6	6	6	6
Mean	45062	44232	28344	27830	4549	4458
SD	85.90	99.04	155.54	170.86	172.72	179.33
% CV	0.19	0.22	0.55	0.61	3.80	4.02
% Mean Recovery	98.16		98.19		98.01	
Overall % Mean Recovery	98.120					
Overall SD	0.0929					
Overall % CV	0.09					

Table 14: Recovery of Cilnidipine

Acquisition Batch ID						
Replicate No.	HQC		MQC1		LQC	
	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response	Un extracted Response	Extracted Response
1	24253	23584	15128	14785	2232	2194
2	23980	23756	15285	14895	2239	2195
3	24296	23889	15059	14783	2388	2345
4	24385	23747	15168	14982	2358	2295
5	24075	23630	15185	14878	2306	2275
6	23879	23490	15092	14786	2259	2216
n	6	6	6	6	6	6
Mean	24145	23683	15153	14852	2297	2253
SD	197.46	142.63	79.82	81.28	64.99	61.52
% CV	0.82	0.60	0.53	0.55	2.83	2.73
% Mean Recovery	98.09		98.01		98.10	
Overall % Mean Recovery	98.066					
Overall SD	0.0474					
Overall % CV	0.05					

Table 14: Recovery of Internal standard

Acquisition Batch ID	Date	
S.No.	Un extracted Area Ratio	Extracted Area Ratio
1	66398	65269
2	66554	65367
3	66680	65486
4	67025	65698
5	66998	65556
6	66685	65410
n	6	6
Mean	66723.3	65464.3
SD	246.70	151.03
% CV	0.37	0.23
% Mean Recovery	98.11	

Stabilities:

Long term stock solution stability for Chlorthalidone: In bench-top stability, six replicates of LQC & HQC samples (0.09 and 0.96 µg/ml) were analyzed for 9 hours at room temperature on the laboratory bench. The % mean stability was calculated and found to 99.80% for LQC and 100.09% for HQC respectively.

Long term stock solution stability for Cilnidipine: In bench-top stability, six replicates of LQC & HQC samples (0.75 and 8.0µg/ml) were analyzed for 9 hours at room temperature on the laboratory bench. The %

mean stability was calculated and found to 99.80% for LQC and 100.32% for HQC respectively.

Matrix samples stability at -28±5 °C for 37 days & -80±5 ^{0}C: Long term stock solution stability for the Chlorthalidone was determined at a concentration of LQC-HQC level after a storage period of 37 days at -28°C& -80°C in refrigerator. The % mean stability of the Chlorthalidone was found to be 101.68%, 99.93% at 28 ± 5°C and 101.31%, 99.89% at 80 ± 5°C respectively.Long term stock solution stability for the Cilnidipine was determined at a concentration of LQC-HQC level after a storage period of 37 days at -28°C& -80°C in refrigerator. The % mean stability of the Cilnidipine was found to be 102.26%, 99.69% at 28 ± 5°C and 102.26%, 100.91 at 80 ± 5°C respectively.

CONCLUSION

A simple, accurate, precise method was developed for the estimation of the Chlorthalidone and Cilnidipine in human plasma using the Azilsartan as internal standard. Retention time of Chlorthalidone and Cilnidipine were found to be 2.860min and 3.385min. %CV of the Chlorthalidone and Cilnidipine were found to be 0.26% and 0.94%. %Recovery was obtained as 98.12% and 98.06%. The linearity concentration is in the range of 0.03 to 1.2 µg /m of Chlorthalidone, 0.015 to 06µg /ml of Cilnidipine .The lower limits of quantification were 0.03µg/mL of Chlorthalidone and 0.015µg/mL of Cilnidipine, which reach the level of both drugs possibly found in human plasma. Further, the reported method was validated as per the ICH guidelines and found to be well within the acceptable range. The proposed method is simple, rapid, accurate, precise, and appropriate for pharmacokinetic and therapeutic drug monitoring in the clinical laboratories.

Acknowledgement: The author expresses sincere thanks to Spectrum research solution, Hyderabad to provide gift samples of clidinipine and Chlorthalidone drug samples. K_2 EDTA control plasma procured by Deccan Pathological labs, Hyderabad.

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