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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CLONIDINE HCL AND HYDROCHLOROTHIAZIDE IN TABLET

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ABSTRACT

Simple, specific, accurate, precise and reproducible and robust method have been developed and validated for the Simultaneous Estimation of Clonidine HCl and Hydrochlorothiazide in tablet. The Reverse Phase High Performance Liquid Chromatography, the chromatographic system was equipped with Kromasil C_{18} column and UV detector set at 254 nm, in conjunction with a mobile phase of 0.1M Sodium Dihydrogen Phosphate buffer and Acetonitrile in the ratio of 70:30 % v/v (pH 3.0, adjusted with 1% orthophosphoric acid) at a flow rate of 1.5 mL/min. The described method was linear over a concentration range of 50-300 µg/mL for Clonidine HCl and Hydrochlorothiazide. The retention time of Clonidine HCl was 5.230 ± 0.26 min and Hydrochlorothiazide was 2.458 ± 0.006 min. The % recoveries of the both the drugs were found to be 99.32 % - 101.55 % and 99.69 % - 101.00 % for Clonidine HCl and Hydrochlorothiazide respectively. Methods were statistically validated for accuracy, precision, specificity, LOD, LOQ and robustness according to ICH guidelines and can be used for analysis of combined tablet formulation. Clonidine HCL and Hydrochlorothiazide were subjected to stressed conditions of acid degradation, base degradation, oxidative degradation and thermal degradation under same chromatographic condition. The stress samples were assayed on RP-HPLC system.

Keywords: Clonidine HCL, Hydrochlorothiazide, RP-HPLC method, Force Degradation Study

INTRODUCTION:

Hypertension (HTN) or high blood pressure, sometimes called arterial hypertension, is a chronic medical condition in which the blood pressure in the arteries is elevated. It is most common disorder affecting the heart and blood vessels. The major cause of heart failure, kidney diseases and stroke **[1]**. Clonidine HCl - N-[(2,6-dichlorophenyl) imino]-imidazolidine hydrochloride is an imidazole group hypertensive agent **[2]** and Hydrochlorothiazide-6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide is thiazide diuretics and it is sulphonamide derivative **[3]**. Structure of Clonidine HCl and Hydrochlorothiazide is shown in **Figure 1 [4-5]**. Clonidine hydrochloride is α_2-Adrenergic receptor agonist. It is an inhibitor of gastrointestinal motility that blocks α_2 sites, which leads to an adrenergic response. Mainly used to treat hypertension. Hydrochlorothiazide, a thiazide diuretic, inhibits water reabsorption in the nephron by inhibiting the sodium-chloride symporter (SLC12A3) in the distal convoluted tubule, which is responsible for 5% of total sodium reabsorption. So, clonidine hydrochloride and hydrochlorothiazide are given into combination **[6-8]**. By the literature survey it was found that analytical methods are available for estimation of Clonidine HCL and Hydrochlorothiazide alone and with other combination. RP-HPLC method was found for estimation of Clonidine HCL and Hydrochlorothiazide in combined dosage form. **[9-15]**. So, there is thought to perform force degradation study of Clonidine HCL and Hydrochlorothiazide in their combined tablet dosage form. With the advent of International Conference on Harmonization (ICH) guidelines, the requirement of establishment of stability -indicating assay method (SIAM) has become more clearly mandated. The guidelines explicitly require conduct of forced decomposition studies under a variety of conditions, like pH, light, oxidation, etc. and separation of drug from degradation products **[16-17]**. Thus, the objectives of this work are to develop a new sensitive stability indicating RP-HPLC method for simultaneous determination of Clonidine HCl and Hydrochlorothiazide. Also, it is validated for market product named Arkamin H containing Clonidine HCl and Hydrochlorothiazide in tablet dosage form **[18]**.

MATERIALS AND METHODS

Standard Clonidine Hydrochloride and Hydrochlorothiazide were obtained as gift

sample from Zydus Pharmaceutical Ltd. and Intas Pharmaceutical Ltd. respectively. Younglin HPLC and YL clarity model 1900 software was used. Acetonitrile, methanol, Sodium dihydrogen phosphate, sodium hydroxide and hydrochloric acid, ortho phosphoric acid of AR grade from S. D. Fine Chemicals, was used. A commercial dosage form ARKAMIN H was purchased from local market.

IR identification and wavelength selection

The individual standard drugs, Clonidine HCl and Hydrochlorothiazide were mixed with KBr and KBr pallets were prepared. These KBr pallets of drugs were used for FTIR analysis. And then FTIR spectra were interpreted and results were co-related with M.P., UV spectra and solubility to confirm identity of individual drugs. Wavelength was selected from the overlay spectra of above solutions.

Selection of Mobile phase

Various mobile phases were tried. Trial contains various mobile phases which consisted of Acetonitrile, methanol, Sodium dihydrogen phosphate, sodium hydroxide and hydrochloric acid, ortho phosphoric acid in different proportions with various pH and different volumes at flow rate 1 ml/min were tried. Chromatogram in optimized mobile phase is shown in Figure.

Preparation of stock solution

Weighed accurately 50 mg of Clonidine HCL and 50 mg of Hydrochlorothiazide was transferred into 50 ml volumetric flask and dissolved into mobile phase and volume was made up to mark (1000 µg/ml).

Preparation of calibration curve

Standard stock solution of Clonidine HCL and Hydrochlorothiazide were further diluted with methanol into 10 ml volumetric flask which contain 50,100,150,200,250,300 µg/ml for both drugs and volume was made up mark with mobile phase. Scan in HPLC and plot the graph of peak area vs concentration. Calibration curve were plotted over a concentration range of 50-300 µg/ml for both drugs.

Preparation of sample solution

Twenty tablets were weighed accurately. Powder equivalent to 20 mg of Hydrochlorothiazide and 0.1 mg of Clonidine HCLwas weighed and transferred in a 100ml volumetric flask. To this flask 19.9 mg of Clonidine HCL was added. 80 ml of mobile phase was added and sonicated for 15 min & filtered to another 100 ml volumetric flask. Volume was made up to mark with mobile phase. 5 ml of resulting solution was transferred to 10 ml volumetric flask and volume was made up to mark with mobile phase.

Optimized Chromatographic Conditions

Parameters	Chromatographic Condition
Mode of elution	Isocratic
Mobile Phase	Acetonitrile: 0.1 M Sodium dihydrogen phosphate pH-3 Adjusted with O-phosphoric acid (1%) (30:70 % v/v)
Column	Kromasil C-18 column (250 mm × 4.6 mm, 5.0µm)
Flow rate	1.5 ml/min
Runtime	12 min
Injection volume	20 µl
Detection wavelength	254 nm

METHOD VALIDATION

Linearity

For the linearity study 5,10,15,20,25,30 ml of Clonidine HCL, 5,10,15,20,25,30 ml of Hydrochlorothiazide was mixed in six 10ml volumetric flask and volume was made up to mark by Methanol. Calibration curve Hydrochlorothiazide and Clonidine HCL are shown in figure.

Repeatability

The data for repeatability of peak area measurement for Clonidine HCL (100 µg/ml) and Hydrochlorothiazide (100 µg/ml) based on six measurements of same solution. The % RSD for Clonidine HCl and Hydrochlorothiazide are shown in table.

Intra-day and inter-day Precision

In the intra-day studies, three repeated injections of standard solution were made and response factor of drug peaks and % RSD were calculated. In inter-day variation studies, three injections of standard solution were made for three consecutive days and response of drug peaks and % RSD were calculated.

LOD and LOQ

Calibration curve was repeated for Three times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where, SD = Standard deviation of intercepts. The results were shown in table.

Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.1 ml/min).
2. pH of Mobile phase was changed (± 0.1).
3. Ratio of Mobile phase was changed (±2).

The results were shown in table.

Assay of marketed formulation

Applicability of proposed method was tested by analysing tablet formulation (Arkamin H). The result is shown in table.

Forced degradation

Acid degradation

Accurately measured 1 ml of standard stock solution of Clonidine HCL and 1 ml of Hydrochlorothiazide were transferred into 10 ml volumetric flask and 1 ml 0.1 N HCl

was added and solution was kept at room temperature for 90 min for acid hydrolysis. Then the solution was neutralized with 0.1 N NaOH and made volume up to mark with diluent to get 100 µg/ml of Clonidine HCL and 100 µg/ml of Hydrochlorothiazide and filtered through 0.45 µm membrane filter paper and injected in to HPLC system.

Base degradation

Accurately measured 1 ml of standard stock solution of Clonidine HCL and 1 ml of Hydrochlorothiazide were transferred into 10 ml volumetric flask and 1 ml 0.1 N NaOH was added and solution was kept at room temperature for 60 min for a base hydrolysis. Then the solution was neutralized with 0.1 N HCl and made volume up to mark with diluent to get 100 µg/ml of Clonidine HCL and 100 µg/ml of Hydrochlorothiazide and filtered through 0.45 µm membrane filter paper and injected in to HPLC system.

Oxidative Degradation

Accurately measured 1 ml of standard stock solution of Clonidine HCL and 1 ml of Hydrochlorothiazide were transferred into 10 ml volumetric flask and 1 ml 3 % H2O2 was added and solution was kept at room temperature for 60 min for an Oxidative hydrolysis and made volume up to mark with diluent to get 100 µg/ml of Clonidine HCL and 100 µg/ml of Hydrochlorothiazide and filtered through 0.45 µm membrane filter paper and injected in to HPLC system.

Thermal degradation

Accurately measured 1 ml of standard stock solution of Clonidine HCL and 1 ml of Hydrochlorothiazide were transferred into 10 ml volumetric flask and solution was heated for 60 min at 70oC for a thermal degradation and made volume up to mark with diluent to get 100 µg/ml of Clonidine HCL and 100 µg/ml of Hydrochlorothiazide and filtered through 0.45 µm membrane filter paper and injected in to HPLC system.

RESULT AND DISCUSSION

The present work aimed development and validation of stability indicating RP-HPLC method for simultaneous estimation of Clonidine HCl and Hydrochlorothiazide. The melting point of Clonidine HCl (308-311 °C) and Hydrochlorothiazide (261-264 °C) was found in the range. Method was developed in mobile phase containing Acetonitrile: 0.1 M Sodium dihydrogen phosphate pH-3 Adjusted with O-phosphoric acid (1%) (30:70 % v/v). Detection was carried out at 254 nm. Method was validated as per ICH guidelines. Linearity and regression data were shown in table and Figure. % recovery was within the range (98% - 102%). Results were shown in table. Hence it is found that the developed method is accurate. %RSD values were <2 for repeatability, intra-day

and inter-day precision. Results were shown in table. So, the developed method was found to be precise. LOD and LOQ values were shown in table. LOD & LOQ confirms the method to be sensitive. Small changes were carried out in mobile phase and flow rate for robustness study, in that % RSD of area was found to be <2. So, the developed method was found to be robust. Various forced degradation conditions were performed in proposed method and it can efficiently separate all the degradation products from the drugs. % degradation values are 5% to 20% degradation of the drug substance, have been considered as reasonable and acceptable for validation of chromatographic assays. So, the developed method is stability indicating.

Table 1: IR spectrum interpretation of Clonidine HCL

Sr. No.	Functional group	Observed value	Standard value
1	-C=C-	1430.46	1630-1660
2	N-C	1030.67	1000-1250
3	C_6H_{12}	1084.54	1040-1100
4	-C-H-	874.49	675-900
5	N-H	3393.52	3300-3500

Table 2: IR spectrum interpretation of Hydrochlorothiazide

Sr. No.	Functional group	Observed value	Standard value
1	N-H	3393.52	3300-3500
2	-C-N	1163.14	1000-1350
3	--CH3	1461.00	1455-1470
4	C_6H_{12}	1056.75	1040-1100
5	-C-H-	738.85	675-900
6	C-Cl	738.85	600-800

Table 3: Selection of mobile phase

Sr. no	Mobile phase composition	Inference
1	Water: Methanol (50:50%v/v)	No peak
2	Water: Methanol (30:70%v/v)	Only one peak (HCTZ)
3	Water: Acetonitrile (50:50%v/v)	Only one peak (HCTZ)
4	Water: Acetonitrile (30:70%v/v)	Only one peak (HCTZ)
5	Acetonitrile: Sodium dihydrogenphosphate buffer (0.1M) (pH 3.0) (50:50% v/v)	Only one peak (HCTZ)
6	Acetonitrile: Sodium dihydrogenphosphate buffer (0.1M) (pH 3.0) (45:55%V/V)	Two peaks were separated but distance between two peaks was too large
7	Acetonitrile: Sodium dihydrogen phosphate buffer (0.1M) (pH 3.0) (40:60%V/V)	Two peaks were separated but distance between two peaks was too large
8	Acetonitrile: Sodium dihydrogen phosphate buffer (0.1M) (pH 3.0) (30:70%V/V)	Peaks were properly separated

Table 4: System suitability studies

Parameters	Clonidine HCL Mean ± SD (n=3)	Hydrochlorothiazide Mean ± SD (n=3)
Retention time (min)	5.230±0.26	2.458±0.006
Theoretical plate	4769±130.23	2323±293.34
Tailing factor	1.727±0.020	1.346±0.02
Resolution	10.66959±0.34	0.00000

Table 5: Specificity Study of Standard

Drug	Concentration (µg/ml)	Standard (Mean ± SD) (n=5)	% RSD
Clonidine HCL	100	7610554 ± 17952.72	0.23
Hydrochlorothiazide	100	4502017 ± 8429.601	0.18

Table 6: Specificity Study of Sample

Drug	Concentration(µg/ml)	Tablet (Mean ± SD) (n=3)	% RSD
Clonidine HCL	100	7616917 ± 12815.6	0.16
Hydrochlorothiazide	100	4487035 ± 7756.961	0.17

Table 7: Linearity study of Hydrochlorothiazide

Concentration (µg/ml)	Peak Area (Mean ± SD) (n=6)	%RSD
50	2680410±5609.103	0.20
100	4948135±36327.97	0.15
150	7762354±41596.74	0.45
200	9500113±63807.48	0.55
250	11685987±218184.3	0.77
300	14091664±59447.59	0.42

Table 8: Linearity study of Clonidine HCL

Concentration (µg/ml)	Peak Area (Mean ± SD) (n=6)	%RSD
50	3525354±45507	1.29
100	7066018±27669.78	0.39
150	10303353±168272.7	1.63
200	13420387±226940.8	1.69
250	16248400±199190.4	1.22
300	19266070±11614.2	1.60

Table 9: Repeatability study

Concentrationof Clonidine HCL (µg/ml)	Clonidine HCL		Concentration of Hydrochlorothiazide(µg/ml)	Hydrochlorothiazide	
	Mean ± SD (n=6)	% RSD		Mean ± SD (n=6)	% RSD
100	11576183±25509.71	0.22	100	10580642±95371.22	0.90

Table 10: Intraday & Interday precision study of Clonidine HCL

Drug	Conc. (µg/ml)	Intra-day precision		Inter-day precision	
		Mean ± SD(n=3)	% RSD	Mean ± SD (n=3)	% RSD
ClonidineHCL	50	7771132±36033.93	0.46	7861925±61824.58	0.78
	100	11681628±183589.7	1.57	11712876±122730.9	1.04
	150	13509048±103845.9	0.77	13509048±160395.6	1.18

Table 11: Intraday & Interday precision study of Hydrochlorothiazide

Drug	Conc. (µg/ml)	Intra-day precision		Inter-day precision	
		Mean ± SD (n=3)	% RSD	Mean ± SD (n=3)	% RSD
Hydrochlorothiazide	50	4576676±34596.63	0.75	4560621±33496.92	0.73
	100	10621059±70370.15	0.66	10645239±100987.5	0.94
	150	13258418±99353.35	0.74	13248719±95077.96	0.71

Table 12: Recovery study

Drug	% Of Level	Amount (µg/ml)	AmountAdded (µg/ml)	Total AmountFound (µg/ml)	% Recovery ± SD (n=3)
Clonidine HCL	50 %	100	50	150	99.32 ± 0.05
	100 %	100	100	200	100.95 ± 0.04
	150 %	100	150	250	101.55 ± 0.27
Hydrochlorothiazide	50 %	100	50	150	99.69 ± 0.11
	100 %	100	100	200	100.11 ±0.06
	150 %	100	150	250	101.00 ±0.02

Table 13: Robustness

Parameter	Value	Area	
		Clonidine HCL	Hydrochlorothiazide
pH (± 0.1)	2.9	7635650	4583894
	3	7610553	4502016
	3.1	7652818	4541742
	Mean ± SD	7633007±17355.53	4541742±33431.44
	% RSD	0.22	0.73
Flow rate (± 0.1)	1.4	7575101	4539774
	1.5	7610553	4502016
	1.6	7671415	4462505
	Mean ± SD	7619023±48712.45	4501432±38637.81
	% RSD	0.63	0.85
Mobile phase (±2.0)	28:72	7634918	4563656
	30:70	7610553	4502016
	32:68	7736786	4451328
	Mean ± SD	7660752±66964.56	4505667±56252.91
	% RSD	0.87	1.24

Table 14: Optical Regression characteristics and validation parameter

Parameter	Clonidine HCL	Hydrochlorothiazide
Calibration Range(µg/ml)	50-300	50-300
Regression equation	Y=62496x+701488	Y=45147x+544019
Slop(m)	62496	45147
Intercept(c)	701488	544019
Correlation co efficient(r)	0.998	0.997
Intraday (% RSD, n=5)	0.46-1.57	0.66-0.75
Interday (% RSD, n=5)	0.78-1.18	0.71-0.94
Accuracy(n=3)	% Recovery ± SD	% Recovery ± SD
(50%)	99.32 ± 0.05	99.69 ± 0.11
(100%)	100.95 ± 0.04	100.11 ±0.06
(150%)	101.55 ± 0.27	101.00 ±0.02
Detection limit (µg/ml)	6.67 µg/ml	5.17 µg/ml
Quantitation limit(µg/ml)	20.21 µg/ml	15.68 µg/ml

Table 15: Analysis of Pharmaceutical dosage form

Formulation	Clonidine HCL			Hydrochlorothiazide		
Arkamin-H	AmountLabeled (mg)	AmountFound (mg)	% Amountfound ± SD (n=3)	AmountLabeled (mg)	AmountFound (mg)	% Amountfound ± SD (n=3)
	0.1	0.10	101.12±0.11	20	20.31	101.57±0.12

Table 16: Optimization of acidic condition

TRIALS	CONDITIONS	INFERENCES	% Degradation	
			Clonidine HCL	Hydrochlorothiazide
1.	0.1 N HCl for 30min.	Degradation wasfound in both drugs in variant percent	2.37%	2.24%
2.	0.1 N HCl for 60min.	Degradation wasfound in both drugs in variant percent	2.44%	6.64%
3.	0.1 N HCl for 90min.	Degradation was Found in bothdrugs in variant percent	2.81%	16.98%

Table 17: Optimization of basic degradation

TRIALS	CONDITIONS	INFERENCES	% Degradation	
			Clonidine HCL	Hydrochlorothiazide
1.	0.1 N NaOH for30 min	Minor degradation was found in both drugs	1.10%	1.23%
2.	1 N NaOH for 30min	Minor degradation was found in both drugs	1.20%	1.71%
3	1 N NaOH for 60min	Minor degradation was found in both drugs	1.26%	2.24%

Table 18: Optimization of oxidation degradation

TRIALS	CONDITIONS	INFERENCES	% Degradation	
			Clonidine HCL	Hydrochlorothiazide
1.	3% H_2O_2 for 30 min	Minor degradation was found	6.16%	8.38%
2.	3% H_2O_2 for 60 min	Degradation found	6.37%	11.12%
3.	3% H_2O_2 for 60 min	Degradation found	6.40%	13.30%

Table 19: Optimization of thermal degradation

TRIALS	CONDITIONS	INFERENCES	% Degradation	
			Clonidine HCL	Hydrochlorothiazide
1	At 60^0C temperature for 60min.	No degradation was found	2.38%	3.78%
2	At 70^0C temperature for 30min.	No degradation was found	3.20%	3.99%
3	At 70^0C temperature for 60min.	Slight degradationinboth drugs was found	3.57%	9.83%

Table 20: Result of force degradation study (Standard)

Condition	% Degradation	
	Clonidine HCL	Hydrochlorothiazide
Acid	1.86 %	16.92 %
Base	1.20 %	1.71 %
Oxidation	6.22 %	13.02 %
Thermal	3.78 %	9.60 %

Table 21: Result of force degradation study (Sample)

Condition	% Degradation	
	Clonidine HCL	Hydrochlorothiazide
Acid	2.81 %	16.98 %
Base	1.26 %	2.24 %
Oxidation	6.37 %	13.30 %
Thermal	3.83 %	9.83 %

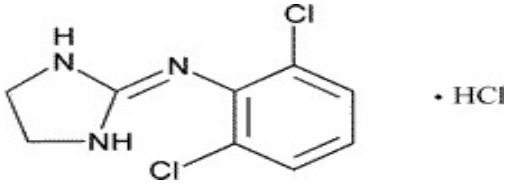

Figure 1. Structure of Clonidine HCL

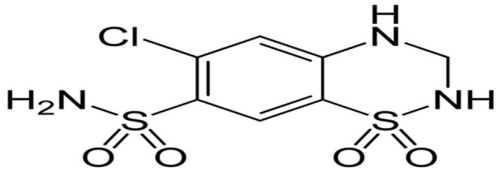

Figure 2. Structure of Hydrochlorothiazide

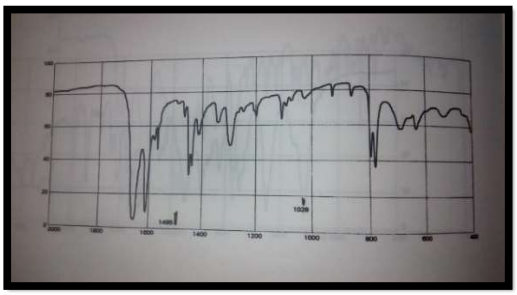

Figure 3: IR spectrum of Clonidine HCL (Std.)

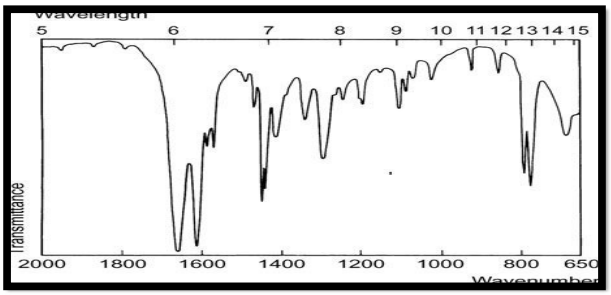

Figure 4: IR Spectrum of Clonidine HCL (API)

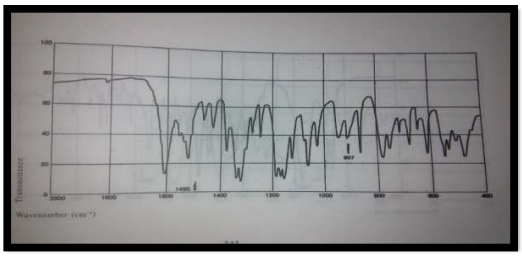

Figure 5: IR spectrum of HCTZ (Std.)

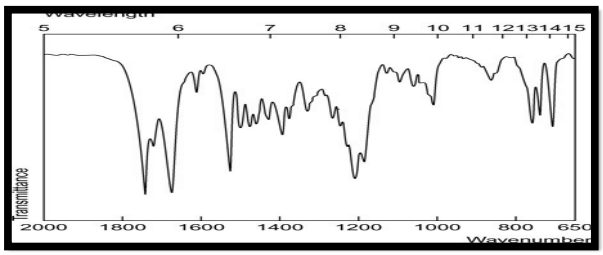

Figure 6: IR Spectrum of HCTZ (API)

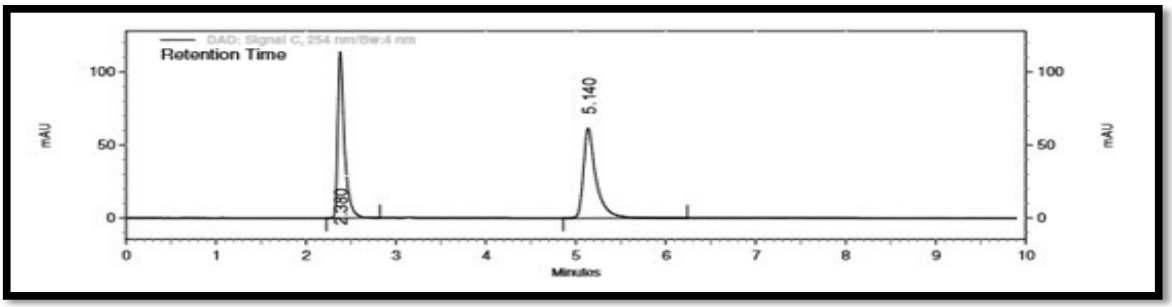

Figure 7: Chromatogram of mixture in Acetonitrile: Sodium dihydrogen phosphate buffer (0.1 M) (pH 3.0) (30: 70%V/V)

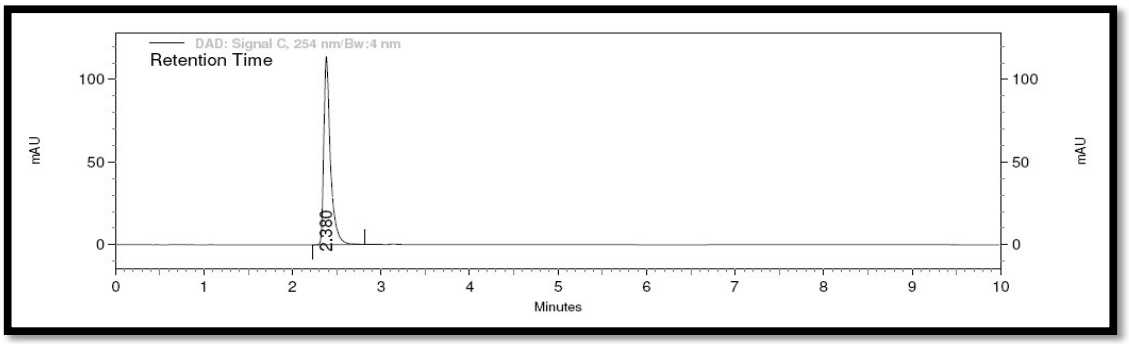

Figure 8: Identification peak of Hydrochlorothiazide

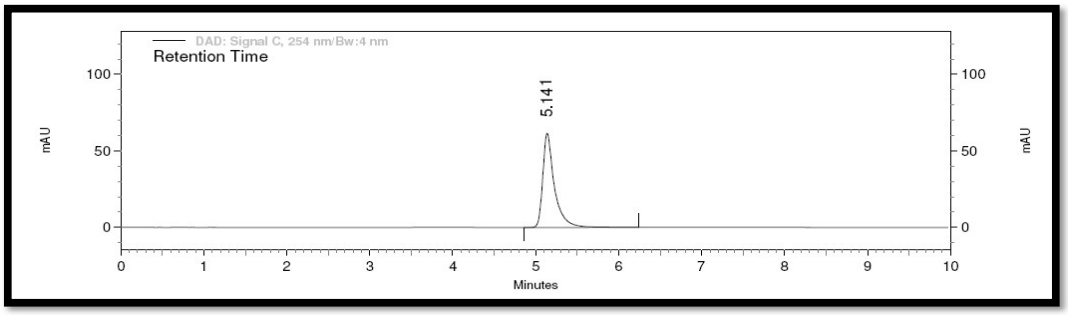

Figure 9: Identification peak of Clonidine HCL

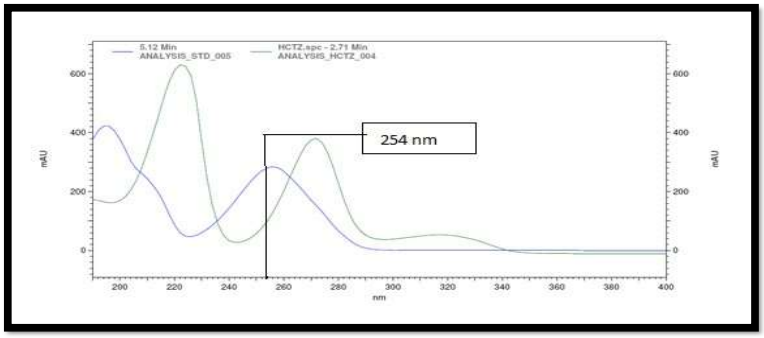

Figure 10: Determination of wavelength maximum

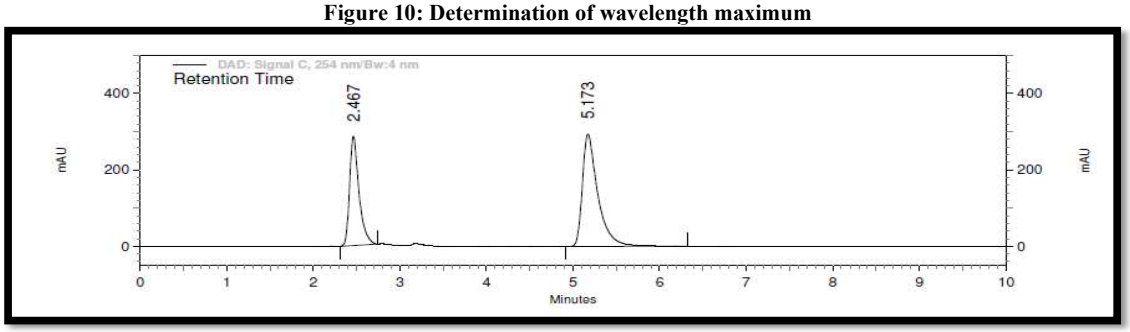

Figure 11: Chromatogram of Standard Hydrochlorothiazide and Clonidine HCL

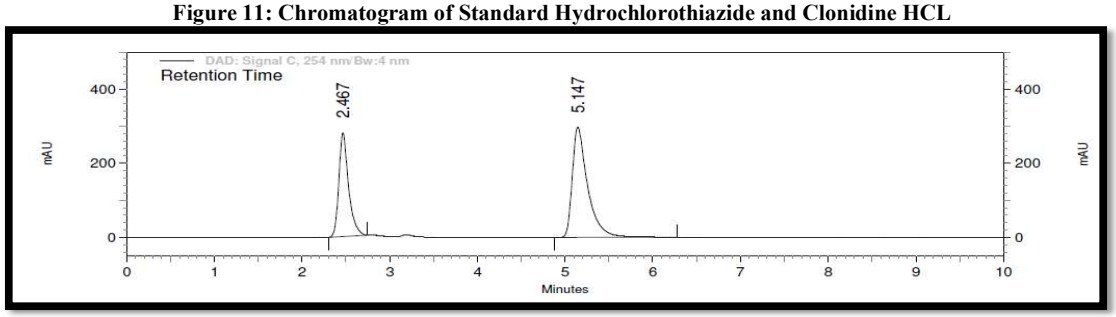

Figure 12: Chromatogram of Sample Hydrochlorothiazide and Clonidine HCL

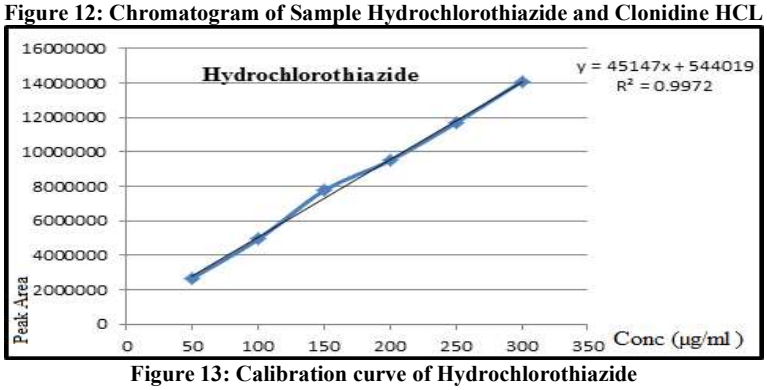

Figure 13: Calibration curve of Hydrochlorothiazide

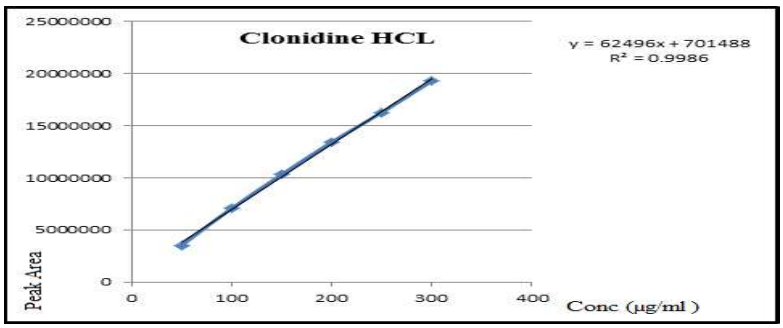

Figure 14: Calibration curve of Clonidine HCL

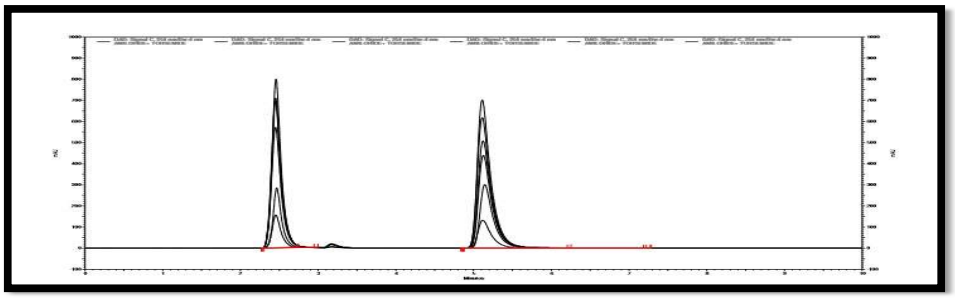

Figure 15: Overlain linearity chromatogram of Hydrochlorothiazide and Clonidine HCL

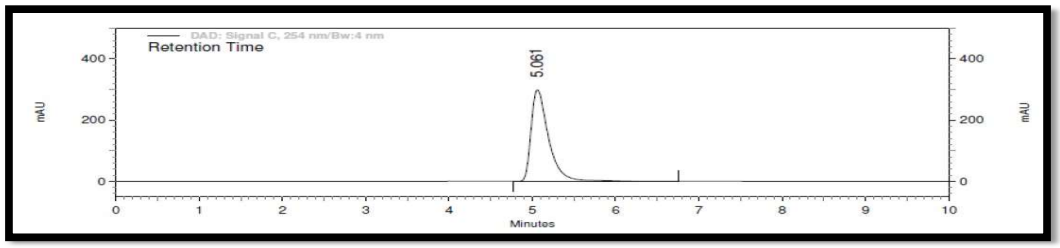

Figure 16: Chromatogram of Clonidine HCL under acid degradation

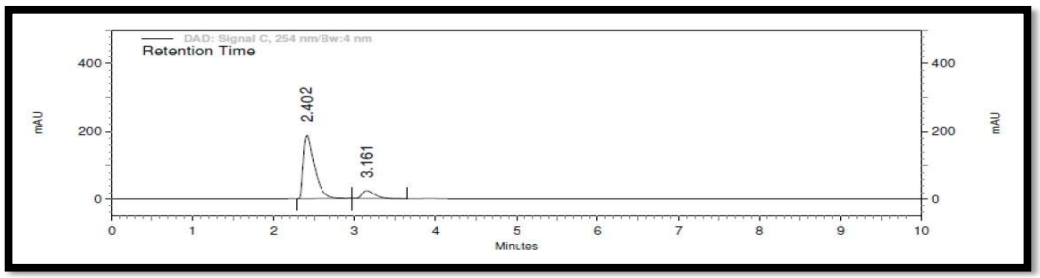

Figure 17: Chromatogram of Hydrochlorothiazide under acid degradation

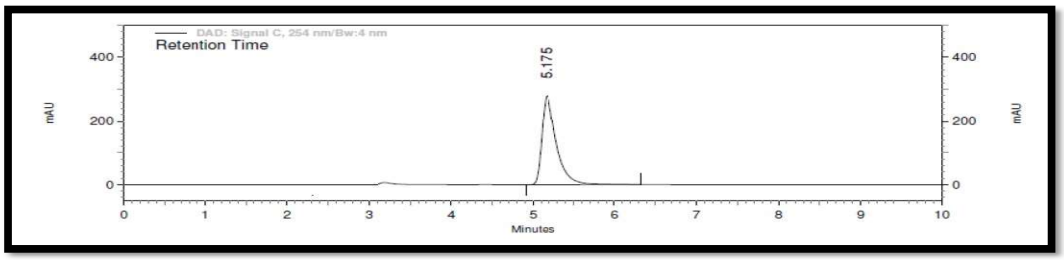

Figure 18: Chromatogram of Clonidine HCL under base degradation

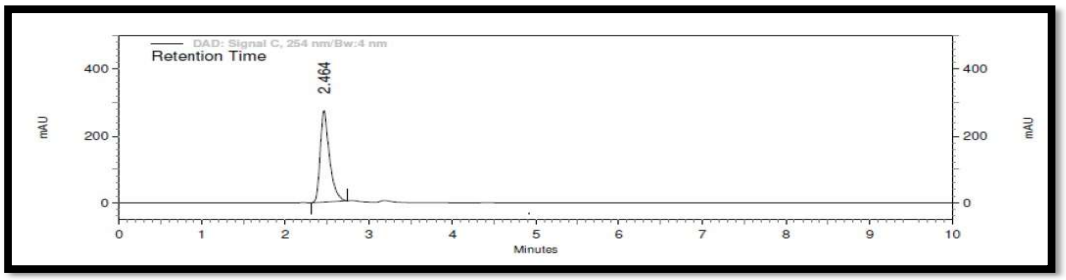

Figure 19: Chromatogram of Hydrochlorothiazide under base degradation

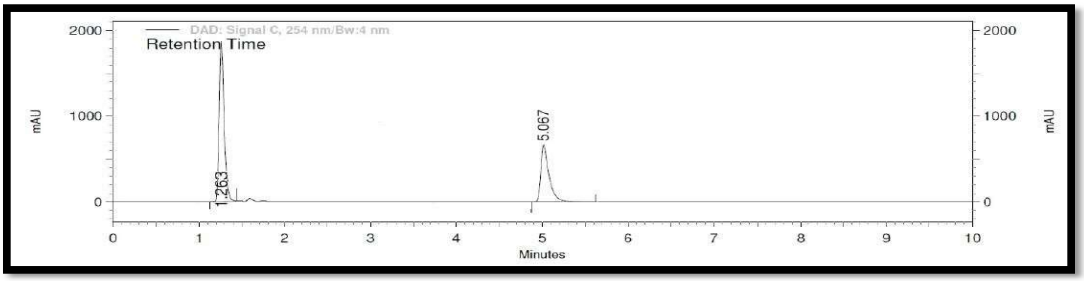

Figure 20: Chromatogram of Clonidine HCL under oxidation degradation

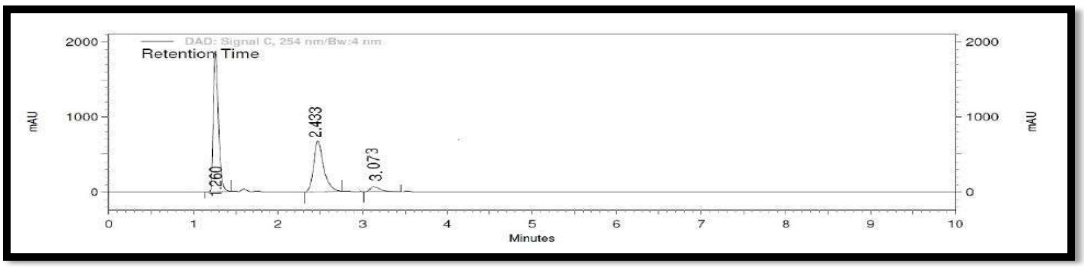

Figure 21: Chromatogram of Hydrochlorothiazide under oxidation degradation

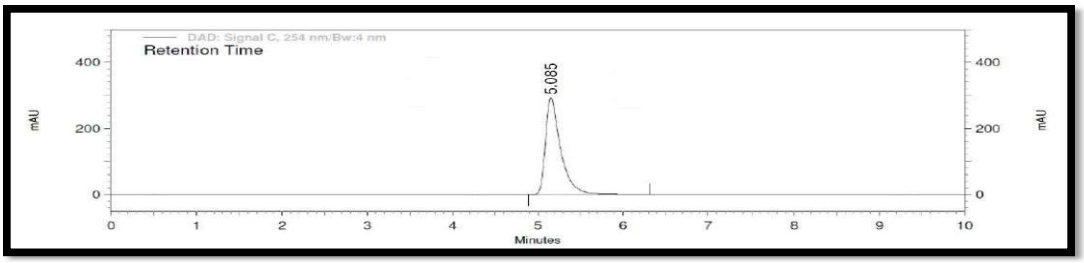

Figure 22: Chromatogram of Clonidine HCL under thermal degradation

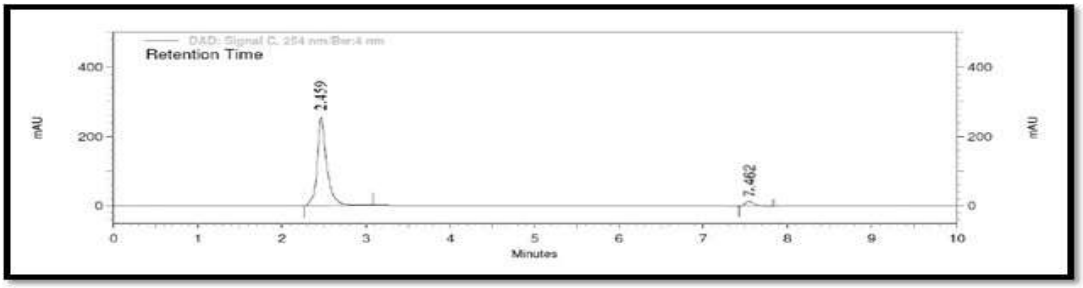

Figure 23: Chromatogram of Hydrochlorothiazide under thermal degradation

CONCLUSION

Clonidine HCL is α_2-Adrenergic receptor agonist. It is an inhibitor of gastrointestinal motility that blocks α_2 sites, which leads to an adrenergic response. Clonidine reduces sympathetic stimulation which leads to lowering of blood pressure and also wing of heart rate. It has been generally proposed that clonidine and related antihypertensive act through a group of receptors called the imidazole receptors. Hydrochlorothiazide a thiazide diuretic, inhibits water reabsorption in the nephron by inhibiting the sodium-chloride symporter (SLC12A3) in the distal convoluted tubule, which is responsible for 5% of total sodium reabsorption. By blocking the sodium- chloride symporter, hydrochlorothiazide effectively reduces the osmotic gradient and water reabsorption throughout the nephron.

RP-HPLC method was developed for simultaneous estimation Clonidine HCL and Hydrochlorothiazide. In RP-HPLC method, good resolution and separation of two drugs was achieved. 0.1 M Sodium dihydrogen phosphate (pH-3): Acetonitrile (70:30 v/v) was used as mobile phase. Retention time of Clonidine HCL and Hydrochlorothiazide were found to be 5.140 and 2.380 min respectively with a flow rate of 1 ml/min. The proposed method was accurate and precise. Therefore, proposed method can be used for routine analysis of Clonidine HCL and Hydrochlorothiazide in tablets.

Forced degradation study of Clonidine HCL and Hydrochlorothiazide was performed by RP-HPLC method which includes Acid, Base, Oxidative and Thermal degradation. Results of degradation were found within limit.

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