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Development And Validation Of Stability Indicating Rp Hplc Method Of Captopril And Hydrochlorothiazide In Bulk And Combine Dosage Form

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Abstract: : A simple, precise, and accurate stability- indicating rear phase high- performance liquid chromatographic(RP- HPLC) system was developed and validated to graphy for the contemporaneous estimation of Captopril and Hydrochlorothiazide in bulk and combined lozenge form. The chromatographic separation was achieved using a C18 column with a suitable mobile phase composed of(insert exact composition, e.g., acetonitrile buffer(pH 3.0) in a defined rate), at a inflow rate of(insert inflow rate) mL/ min and discovery wavelength at(insert discovery wavelength, e.g., 220 nm). The system was validated as per ICH guidelines for parameters including linearity, delicacy, perfection, particularity, limit of discovery(LOD), and limit of quantification(LOQ). Forced declination studies were conducted under colorful stress conditions like acid, base, oxidation, thermal, and photolytic to establish the stability- indicating nature of the system. Both medicines were well resolved with satisfactory retention times, and no hindrance from declination products was observed. The developed RP- HPLC system proved to be dependable, robust, and suitable for routine quality control analysis of Captopril and Hydrochlorothiazide in both bulk medicine and pharmaceutical phrasings.

Index Terms : Accuracy, bulk drug , captopril, combine dosage form ,force degradation ,hydrochlorothiazide ,ich guideline ,linearity ,method development, method validation, pharmaceutical analysis, precision, retention time ,rp-hplc, specificity &stability indicating method.

I . INTRODUCTION:

High performance liquid chromatography (HPLC) chromatography is a process which separate chemical species from one another abecedarian driving force chromatography is the chemical equilibrium that affect species distribution between two phases. Chromatographic Process:- In the chromatographic process species distribute between two immiscible phases one phase is following and the other is stationary. Stationary phase are composed of nearly spaced pervious flyspeck packed into a tube called as column. the mobile phase fill the spaced between the flyspeck. Common detergent 1. Hexane 2. Dichloromethane 3. Methyl t- butyl – ether 4. Ethyl acetate 5. Chloroform 6. Tetra hydro furan 7. Acetonitrile 8. Methanol 9. Water. There are three introductory type of laboratory columns. Microborer Diameter 1- 2 mm Length 7- 30 cm Sample size 0.01 mg Flow rate 0.1 ml/ min. Standard : Diameter : 3-5mm Length : 7-30cm Sample size : 0.1mg Flow rate : 1.0ml/min C .Preparative : Diameter : 5-20mm Length : 25-50cm Sample size : 10mg Flow rate : 10ml/min. Chromatography: The liquid chromatograph consist of several component either assembled as⎫ shown here or integrated into single unit. Each of these component will be . UV Sensor :- The variable wavelength UV sensor is the most popular HPLC sensor.⎫ Ultraviolet light from a deuterium beacon is resolve into its element⎫ wavelengths by a grating mono chromate cadence. By rotating the grating, a single wavelength is named at which the sample⎫ absorbs. Exit gashes allow only the named wavelength to pass through the

inflow⎫ cell. The intensity of the transmitted ray is measured with a UV Sensor Advantages϶ 1) perceptivity Variable wavelength UV sensors are able of handling wavelengths in the range of 190 – 350nm. While not all emulsion absorb in this range, the vast maturity do. For composites that do absorb, the perceptivity of the UV sensor is excellent. attention down to a bit or a part- per- million(ppm) can generally be measured. 2) Selectivity When peaks lap, the selectivity of the UV sensor can be employed. If the snooping factors have a UV diapason different from the element of interest, a wavelength can be named which minimize the hindrance. 3) grade UV sensors can be used for grade elution, furnishing that the eluents do not absorb at the wavelength being covered. Method Development 1.) Sample Preparation 2.) Column Selection 3.) Sensor Selection 4.) Eluent Survey 5.) Optimization Sample PEAK Dimension of the peak height with a sovereign is the⎫ simplest fashion. Alternately, dimension of the area of the triangle drawn through the peak curve points can ameliorate delicacy. If an integrator or data system is used, the electronic integration⎫ parameters must be acclimated similar that nascence's are drawn duly and start and stop points of the integration are located rightly. The launch point must be set where the peak just begins to rise above⎫ the birth. The stop point must not go beyond where the response returns to the birth Estimation should be done daily. Four to six standard results gauging the attention range anticipated for⎫ the samples should be run. A estimation wind is also constructed by conniving attention versus⎫ the peak height or area. If the estimation wind is set up to be direct, and the line passes through the origin, diurnal estimation can be fulfilled with only one or two norms. chromatography system General bracket Gas chromatography (GC) , Liquid chromatography(LC) ,Super critical fluid chromatography . 2. Specific system Gas a) liquid chromatography(GLC) b) Gas – liquid Liquid – liquid or partition Liquid – c) solid or adsorption d) Ion exchange e) Size rejection f) Affinity g) Separation and sanctification.) Stationary phase Liquid a) adsorbed or clicked to a solid face Solid b) Ion exchange c) resin d) Liquid in interstices of a polymeric e) solid Group specific f) liquid clicked to a solid face Organic species clicked to a solid face. Type of equilibrium Partition a) between gas and liquid b) Adsorption c) Partition between immiscible d) liquid Adsorption e) Ion exchange f) Partition/ sieving Partition g) between face liquid and mobile h) liquid Partition between super critical fluid i) and clicked face. System element1) Solvent delivery system- The most important element of HPLC in solvent delivery system pump,⎫ because is performance directly prompt the retention, time reproducibility and sensor perceptivity, among the several solvent delivery system(direct gas pressure, curvaceous intensifier, repaying pump with Two system of optimization 1. Homemade 2. Computer driven selection of sensor :- 1. Mode of selection- :- The rear phase mode, the mobile phase is comparatively more polar thane⎫ the stationary phase. for the separation of polar or relatively polar composites, the alternate factor is the nature of the matrix. 2. Selection of mobile phase- :- In the liquid chromatography, the solute retention is governed by the solute⎫ distribution factor, which reflect the different injection of the solute. the mobile phase the nature composition of which has to be⎫ judiciously named in order to get applicable and required solute retention. Analytical parameter :- 1. particularity/ selectivity 2. System felicity 3. Precision 4. Repeatability 5. Inter intervene perfection Reproducibility 6. delicacy 7. Linearity 8. Range 9. Limit of discovery 10. Limit of quantitation 11. Robustness 12. Stability study. Medicine profile Captopril-, Structure- Molecular formula :- C9H15NO3S, Molecular weight :- 217.29 g/ spook, Chemical name :- 1-(2 methyl-3, sulphonylpropanoyl)pyrolidine-2 – carboxylic acid. Color :- white to off – crystalline greasepaint, Melting point :- 103 – 104 osmolality :- freely answerable in water,Vapourpressure :- 7.25 x 10 – 6mmhg• order :- Anti hypertensive medicine• Odor :- slight sulfa•) Hydrochlorothiazide Molecular formula :- C7H8CIN3O4S2 • Molecular weight :- 297.7 g/ spook ,Chemical name:-6 – choro -3,4 dihydro-2 H – benzothiadiazide 7 – sulfonamide 1,1 – dioxide. ,Color white :- crystalline greasepaint • Melting point:- 274 oC • Solubility :- lower than 0.1 mg/ ml • Vapour pressure :- 1.78 x 10 – 10 mmhg • order :- Anti hypertensive agent ,Odour :- odourless Pka :- 7.9, 9.2.

II . Aim and objective

Aim-

To develop a new stability indicating .

RP –HPLC system for contemporaneous estimation of captopril and hydrochlorothiazide in bulk and combined lozenge form.

Ideal :- To develop simple, new, rapid-fire, reproducible system for the anti⎫ hypertensive class of lozenge form. To develop accurate, precise, specific, sensitive, and picky system for⎫ quantification of antihypertensive class in tablet lozenge form . To validate the advanced system as per the ICH guideline and verification⎫ of its felicity for used. A rapid-fire and

SENSITIVE REAR- PHASE HIGH PERFORMANCE LIQUID⎫ CHROMATOGRAPHY(RP –HPLC) SYSTEM WITH ULTRA-VIOLET(UV).

DISCOVERY FOR A ROUTINE CONTROL OF HYDROCHLOROTHIAZIDE AND CAPTOPRIL IN⎫ TABLETS WAS DEVELOPED. THIAZIDES INCREASE THE RE ABSORPTION OF CALCIUM IN THIS MEMBER IN A MANNER UNCONNECTED TO SODIUM TRANSPORT ALSO, BY OTHER MECHANISMS, HCTZ IS BELIEVED TOLOWER SUPPLEMENTAL⎫ VASCULAR RESISTANCE. FORCED DECLINATION IS A DECLINATION OF NEW MEDICINE SUBSTANCE E AND⎫ MEDICINE PRODUCT AT CONDITIONS MORE SEVERE THAN ACCELERATED CONDITIONS. O IT'S NEEDED TO DEMONSTRATE PARTICULARITY OF STABILITY INDICATING STYLES.

III. Plan of work

IT HAD BEEN PLANED TO DEVELOP AND VALIDATE REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE SIMULTANEOUS ESTIMATION OF CAPTOPRIL AND HYDROCHLOROTHIAZIDE . 1) LITERATURE SURVEY 2) SELECTION OF DRUG 3) PROCUREMENT OF DRUG 4) STUDY OF PHYSICAL PROPERTIES OF DRUG 5) SOLUBILITY & DETERMINATION OF DRUG 6) SELECTION OF WAVELENGTH (Λ MAX) 7) OPTIMIZATION OF ANALYTICAL METHOD 8) VALIDATION OF DEVELOPED METHOD A) ACCURACY B) PRECISION C) LINEARITY D) RANGE E) LIMIT OF DETECTION F) LIMIT OF DETECTION G) RUGGEDNESS H) ROBUSTNESS. SELECTION OF MOBILE PHASE 2) SELECTION OF CHROMATOGRAPHIC CONDITION . 3) SPECIFICITY 4) STABILITY STUDY . STEPS IN METHOD DEVELOPMENT :- PREPARATION OF STANDARD STOCK .⎫ PREPARATION OF SAMPLE SOLUTION⎫ SELECTION OF INTERNAL STANDARD⎫ SELECTION AND OPTIMIZATION OF MOBILE PHASE .⎫ SELECTION OF CHROMATOGRAPHY CONDITION .⎫ • ISOCRATIC OR GRADIENT ANALUEIS . • COLUMN . • COLUMN & SAMPLE TEMPERATURE • FLOW RATE • INJECTION VOLUME • SET WAVELENGTH.

IV. MATERIAL AND CHEMICAL

I. MATERIAL :- CAPTOPRIL ,HYDROCHLOROTHIAZIDE ACETONITRILE , PHOSPHATE BUFFER , METHANOL ,POTASSIUM D DEHYDROGENATE ORTHO PHOSPHATE BUFFER , ORTHOPHOSPHORIC ACID . SOURCE :- SIGMA – ALDRICH INDIA . INSTRUMENTS :-

HPLC INSTRUMENT UV – SPECTROPHOTOMETER COLUMN , PH METER, BALANCE O SONICATOR NYLON FILTER PAPER.

II. EXPERIMENTAL WORK / RESEARCH METHODOLOGY

TRAIL 1 :- METHANOL (80% + 20 %) WATER WAVE LENGHT 238 FLOW RATE 0.7 COLUMN 100 4.6) SAMPLING MOBILE PHASE

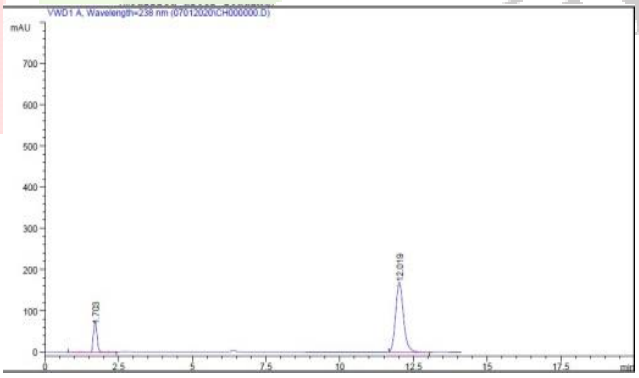


FIG : 1 CHROMATOGRAM TRAIL 01

RETENTION TIME (MIN)	K'	AREA MAU *S	HEIGHT (MAU)	SYMM	WIDTH (MIN)	PLATES	RESOLUTION	SELECTIVITY
1.703	-	649.92566	61.72150	1.00	0.1505	709	-	-
12.019	-	3137.83	165.1880	0.82	0.2815	10100	28.06	7.06

TRIAL 2 :- METHANOL(40% + 60 % WATER WAVW LENGHT 238 FLO W RATE 0.7 COLUMN 100 4.6) SAMPLING MOBILE PHASE.

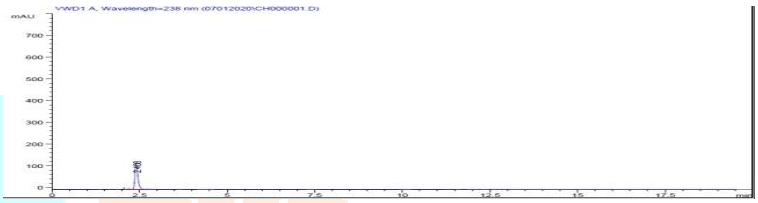


TABLE NO. 02 . DETAIL OF CHROMATOGRAM OF STANDARD MIXTURE CONTAINING

RATE TIME (MIN)	K,	AREA MAU *S	HEIGHT (MAU)	SYMM	WIDTH (MIN)	PLATES	RESOLUTION	SELECTIVITY
2.408	-	701.67450	69.1652	0.85	0.1181	2303	-	-

FROM THE ABOVE ANALYSIS OF TRAIL 02 RESULT IT WAS CONCLUDED THAT NO SHARP PEAK FOR CAP 2.408 MIN .WAS OBTAINED HENCE THE TRAIL WAS REJECTED.

TRIAL 3 :-

METHANOL(70% + 30 % WATER WAVW LENGHT 238 FLOW

RATE 0.7 COLUMN 100 4.6) SAMPLING MOBILE PHASE

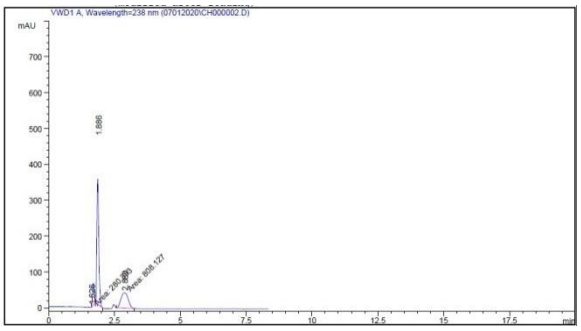


FIG : 3 CHROMATOGRAM TRAIL 03

TABLE NO. 03 DETAIL OF CHROMATOGRAM OF STANDARD MIXTURE CONTAINING CAPTOPRIL AND HYDROCHLOROTHIAZIDE .

RETENTION TIME (MIN)	K,	AREA MAU *S	HEIGHT (MAU)	SYMM	WIDTH (MIN)	PLATES	RESOLUTION	SELECTIVITY
1.626	-	280.352	3.16625	0.00	-	-	-	-
1.886	-	1780.393	465.846	0.95	0.0590	5653	-	1.16
2.880		808.126	43.200	1.09	0.3058	491	3.20	1.53

TRIAL 4 :-

METHANOL (70% + 30 % WATER) WAVE LENTH 238 FLO

W RATE 0.7 COLUMN 100 4.6) SAMPLING MOBILE PHASE-2

30 MCG.

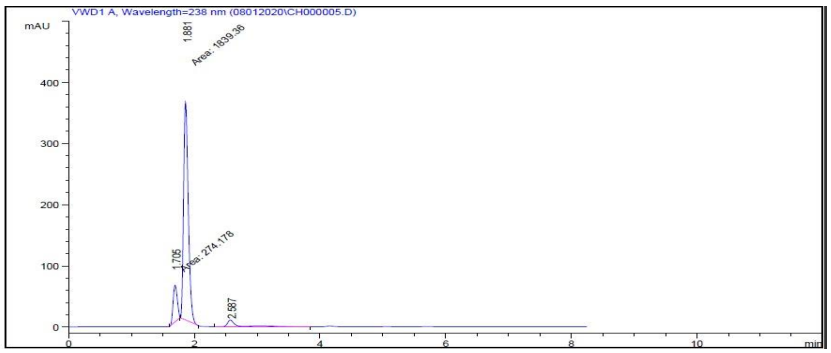


FIG : 4 CHROMATOGRAM TRAIL 04

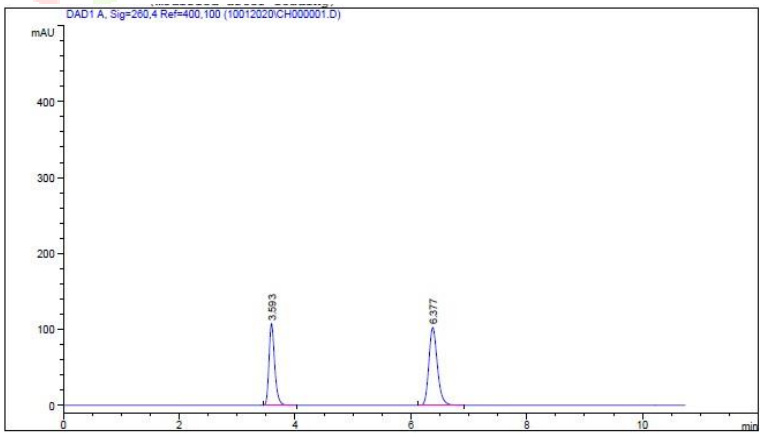
TABLE NO. 04 . DETAIL OF CHROMATOGRAM OF STANDARD MIXTURE CONTAINING CAPTOPRIL AND HYDROCHLOROTHIAZIDE

RETENTION TIME (MIN)	K'	AREA MAU *S	HEIGHT (MAU)	SYMM	WIDTH (MIN)	PLATES	RESOLUTION	SELECTIVITY
1.705	-	274.1784	79.51815	0.47	0.0565	5049	-	-
1.881	-	1839.3584	471.1570	0.72	0.0607	5328	1.77	1.10
2.587	-	139.051	8.8659	0.30	0.1213	2518	4.55	1.37

FROM THE ABOVE ANALYSIS OF TRAIL 01 RESULT IT WAS CONCLUDED THAT NO SHARP PEAK FOR CAP 1.705 MIN .

WAS OBTAINED HENCE THE TRAIL WAS REJECTED.

CAP+HCT :- (70% WATER+30% MEOH)10 MCG-01



Chromatogram final trail

Table no. 01. Detail of chromatogram of standard mixture containing captopril and hydrochlorothiazide .

Retention time (min)	K'	Area Mau *s	Height (Mau)	Symm	Width (min)	Plates	Resolution	Selectivity
3.593	-	736.19934	107.55623	0.75	0.1017	6918	-	-
6.377	-	1047.80090	102.51885	0.79	0.1517	9793	12.91	1.77

From the above analysis of final trail both of result it was concluded that sharp peak for CAP , 3.593 min. and HCT ,6.377 was obtained hence the trail was selected .

2 CAP+HCT :- (70% WATER+30% MEOH) 10MCG-01

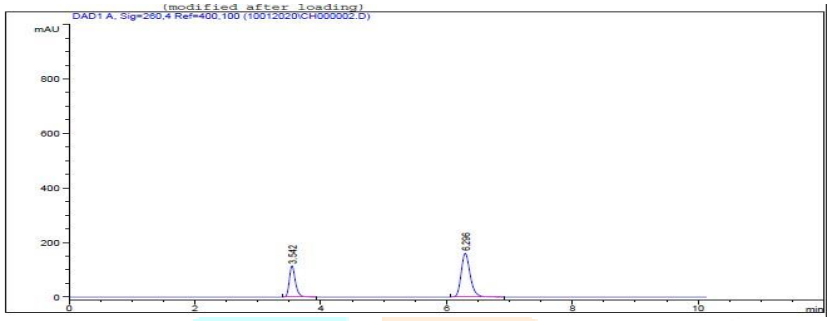


FIG : CHROMATOGRAM FINAL TRAIL

TABLE NO. 02 . DETAIL OF CHROMATOGRAM OF STANDARD MIXTURE CONTAINING CAPTOPRIL AND HYDROCHLOROTHIAZIDE .

RETENTION TIME (MIN)	K'	AREA MAU *S	HEIGHT (MAU)	SYMM	WIDTH (MIN)	PLATES	RESOLUTION	SELECTIVITY
3.542	**-**	**764.87115**	**114.64276**	**0.74**	**0.0973**	**7337**	**-**	**-**
6.296	**-**	**1596.40002**	**160.55089**	**0.78**	**0.1467**	**10210**	**13.26**	**1.78**

FROM THE ABOVE ANALYSIS OF FINAL TRAIL RESULT IT WAS CONCLUDED THAT SHARP PEAK FOR CAP 3.542 MIN. AND HCT 6.296MIN . WAS OBTAINED HENCE THE TRAIL WAS SELECTED

SAMPLE PREPARATION :-

STANDARD SAMPLE PREPARATION- :-

1) **TAKE 0.5 ML STOCK I AND MAKE 10 ML WITH M.P =50 µG/ML HCZ& 100 µG/ML CAPT..**

V. RESULTS AND DISCUSSION

2) STD .HCZ 10 MG AND CAPT. 20 MG IN 10 ML MEOH = 1000 µGM/ML HCZ & 2000 µG/ML CAPT..----- STOCK-1

3) TAKE 0.1 ML STOCK I AND MAKE 10 ML WITH M.P = 10 µG/ML HCZ& 20 µG/ML CAPT.

4) TAKE 0.2 ML STOCK I AND MAKE 10 ML WITH M.P = 20 µG/ML HCZ& 40 µG/ML CAPT..

5) TAKE 0.3 ML STOCK I AND MAKE 10 ML WITH M.P = 30 µG/ML HCZ&60 µG/ML CAPT..

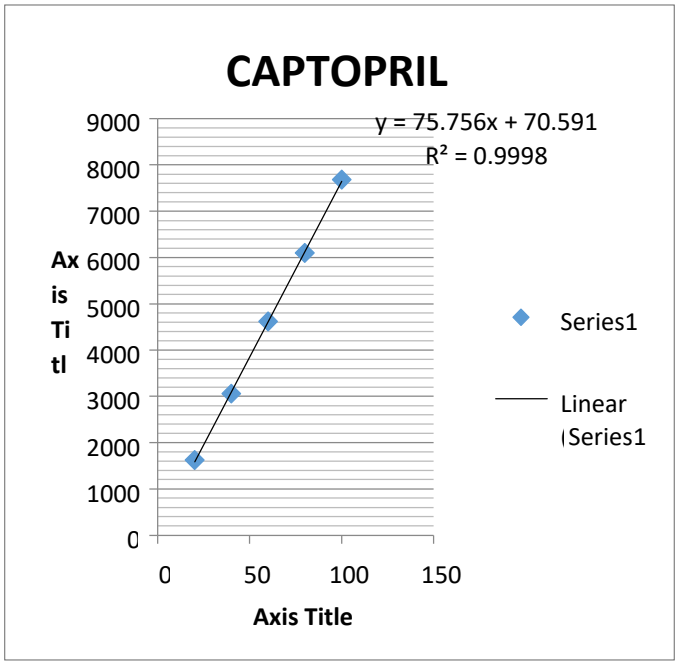
6) TAKE 0.4 ML STOCK I AND MAKE 10 ML WITH M.P = 40 µG/ML HCZ& 80 µG/ML CAPT..

The result of RP- HPLC system was developed for contemporaneous estimation Captopril and Hydrochlorothiazide in bulk and tablet lozenge form.
The separation was achieved by C18 Grace column of × 100 mm with flyspeck size quilting 2.5 µm and Methanol Water(6040 v/ v) pH 3 with OPA(ortho phosphoric acid) as mobile phase at a inflow rate of 1.0 ml/ min.
The discovery was carried out at 205 nm. The retention time of Captopril and Hydrochlorothiazide was set up to be 3.593 ± 3.542 min and 6.377 ± 6.296 min independently. After establishing the chromatographic conditions, analysis of tablet expression was done.
The results are given in, table System felicity Parameters Proposed Method CAP HTZ Retention time 3.542 6.296 Area 764.87115 1596.40002 Theoretical plate 7337 10210 trailing factor- 1.78

System Suitability Parameters	Proposed Method	
	CAP	HTZ
Retention time	**3.542**	**6.296**
Area	**764.87115**	**1596.40002**
Theoretical plate	**7337**	**10210**
Tailing factor	**-**	**1 .78**

system of confirmation-
1)Linearity :

Sr. no.	Concentration	Area
1	20	1623.19
2	40	3057.91
3	60	4621.18
4	80	6099.32
5	100	7678.05



Calibration graph of Captopril.

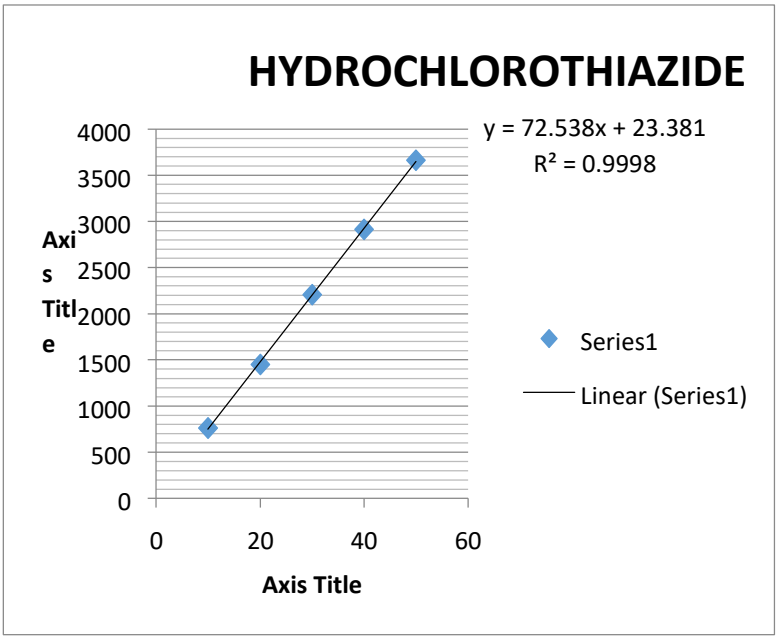
Equation	**Y= 75.75 x + 70.59**
Pitch	70.59
Intercept	75.75 c
Regration	0.999

Linearity of captopril :-

Conc	**Area I**	**II**	**III**	**IV**	**V**	**Mean**	**SD**	**%RSD**
20	1628.44	1617.93				1623.19	7.43	0.46
40	3047.88	3067.94				3057.91	14.18	0.46
60	4623.58	4618.78				4621.18	3.39	0.07
80	6107.77	6090.87				6099.32	11.95	0.20
100	7671.39	7684.7				7678.05	9.41	0.12

Linearity of Hydrochlorothiazide :-

Sr no.	**Concentration**	**Area**
1	10	763.81
2	20	1452.45
3	30	2207.37
4	40	2914.1
5	50	3659.89



Linearity of hydrochlorothiazide

Equation	**Y=72.53X +23.38**
Pitch	72.53m
Intercept	23.38 c
Regration	0.999

Linearity of hydrochlorothiazide :

Sr No.	**Conc**	**Area I**	**II**	**III**	**IV**	**V**	**Mean**	**SD**	**%RSD**
1	10	764.66	762.95				763.81	1.21	0.16
2	20	1449.91	1454.99				1452.45	3.59	0.25
3	30	2208.47	2206.27				2207.37	1.56	0.07
4	40	2919.3	2908.9				2914.10	7.35	0.25
5	50	3658.34	3661.43				3659.89	2.18	0.06
							Avrg SD	3.18	

Linearity :-

a) 10 + 20 MCG 01 :-

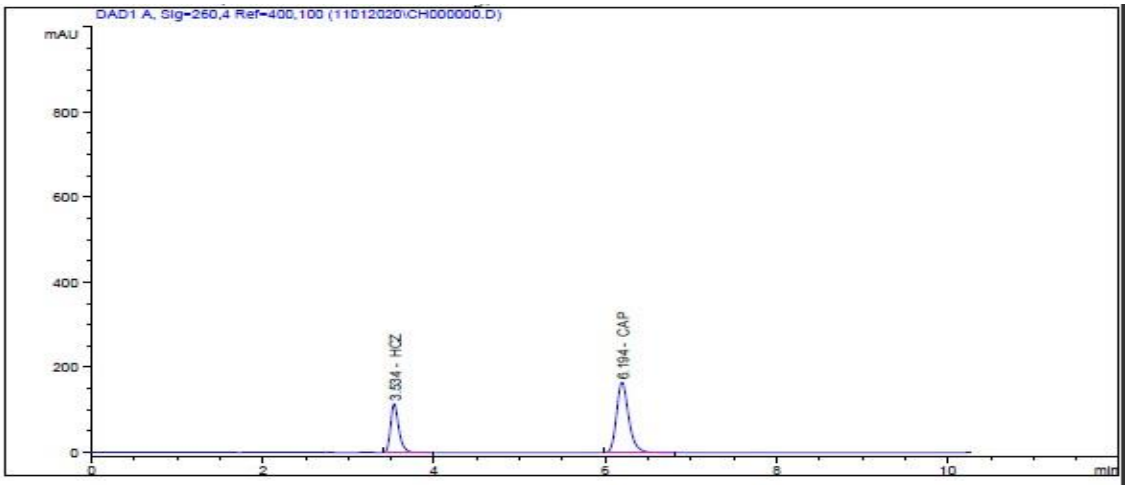


Fig chromatogram of linearity 01

Retention time (min)	K'	Area Mau *s	Height (Mau)	Symm	Width (min)	Plates	Resolution	Selectivity
3.534	-	764.66620	114.74021	0.73	0.0983		-	-
6.194	-	1628.44727	164.98015	0.76	0.1467	9881	12.76	1.75

a) 10 + 20 MCG 02 :-

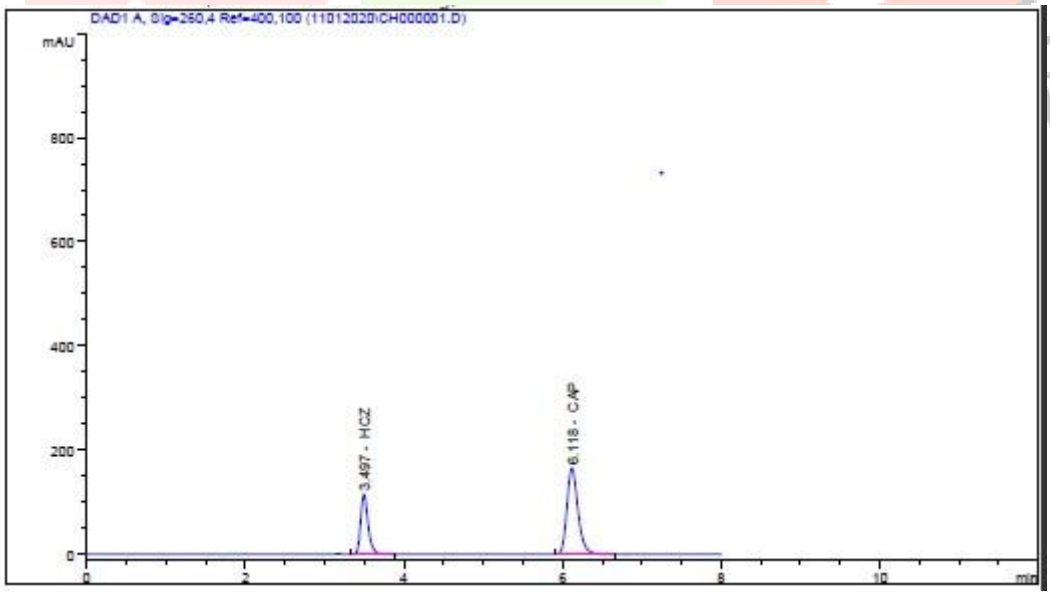


Fig chromatogram of linearity 02

Retention time (min)	**K'**	**Area Mau *s**	**Height (Mau)**	**Symm**	**Width (min)**	**Plates**	**Resolution**	**Selectivity**
3.497	-	762.95868	115.78313	0.73	0.0960	7351	-	-
6.118	-	1617.93005	165.71230	0.76	0.1450	9864	12.78	1.75

a) 20 +40 MCG 01 :-

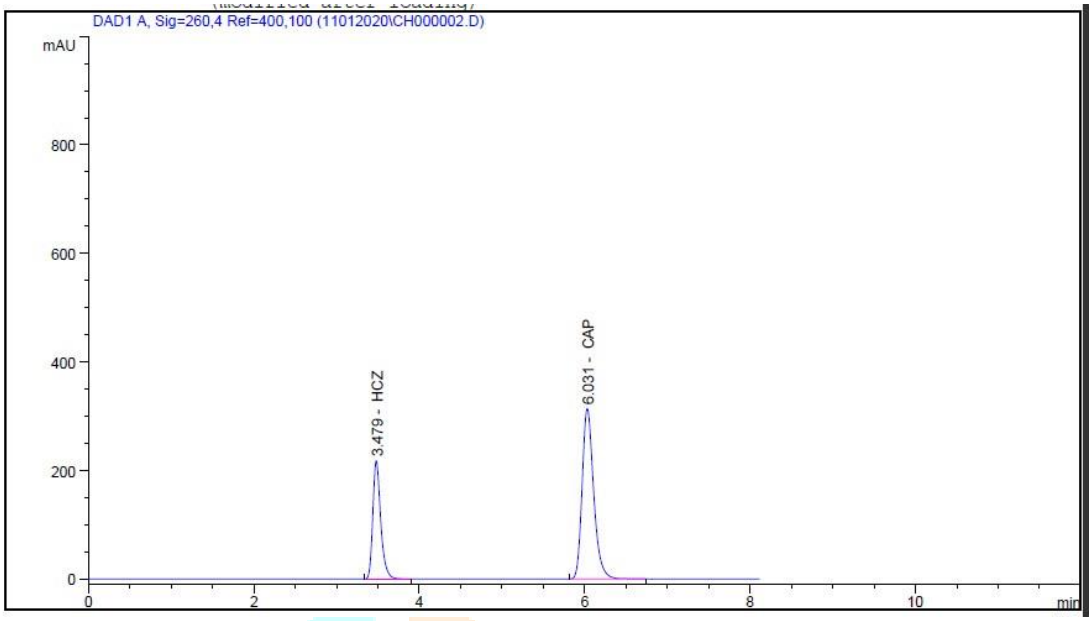


Fig chromatogram of linearity 01

Recovery Study of captopril :-

		0%	**ACCURACY**				
µgm/ml	**Amt added**	**Area**	**Amt found**		**AMT RCVD**		**% RCVD**
TABLET	**API**	**Area**	**AREA±**	**/**	**AMT FOUND**	**AMTRCVD**	*****
SOLUTIN	**SOLUTION**		**INTERCPT**	**SLOPE**	**MINUS**	**DEVIDED**	**100**
					TABLET	**API SOL**	
					SOLUTION		
			80%	**ACCURACY**		**Recovery Study**	

Sr no.	**µgm/ml**	**Amt added**	**Area**	**Amt found**	**Amt recvd**	**% Recv**
1	**20**	**16**	**2807.93**	**36.14**	**16.14**	**100.85**
2	**20**	**16**	**2808.31**	**36.14**	**16.14**	**100.88**
			Mean	**36.14**	**16.14**	**100.87**
			SD	**0.02**	**0.01**	**0.02**
			%RSD	**0.03**	**0.02**	**0.02**
			100%			
Sr no.	**µgm/ml**	**Amt added**	**Area**	**Amt found**	**Amt recvd**	**% Recv**

1	20	20	3106.84	40.08	20.08	100.41
2	20	20	3103.23	40.03	20.05	100.17
			Mean	40.06	20.07	100.29
			SD	0.04	0.02	0.17
			%RSD	0.09	0.11	0.17
			120			
Sr no.	µgm/ml	Amt added	Area	Amt found	Amt recvd	% Recv
2	20	24	3408.83	44.07	24.07	100.29
			Mean	44.03	24.03	100.13
			SD	0.06	0.06	0.23
			%RSD	0.13	0.24	0.23

Recovery Study of hydrochlorothiazide :-

			0%	ACCURACY				
Sr no.	µgm/ml	Amt added	Area	Amt found		AMT RCVD		% RCVD
	TABLET	API	Area	AREA±	/	AMT FOUND	AMTRCVD	*
	SOLUTIN	SOLUTION		INTERCPT	SLOPE	MINUS	DEVIDED	100
						TABLET	API SOL	
						SOLUTION		

			80%					
Sr no.	µgm/ml	Amt added	Area	Amt found		Amt rcvd		% rcvd
1	10	8	1332.35	18.05		8.05		100.59
2	10	8	1336.57	18.11		8.11		101.32
			Mean	18.08		8.08		100.96
			SD	0.04		0.04		0.52
			%RSD	0.23		0.53		0.51

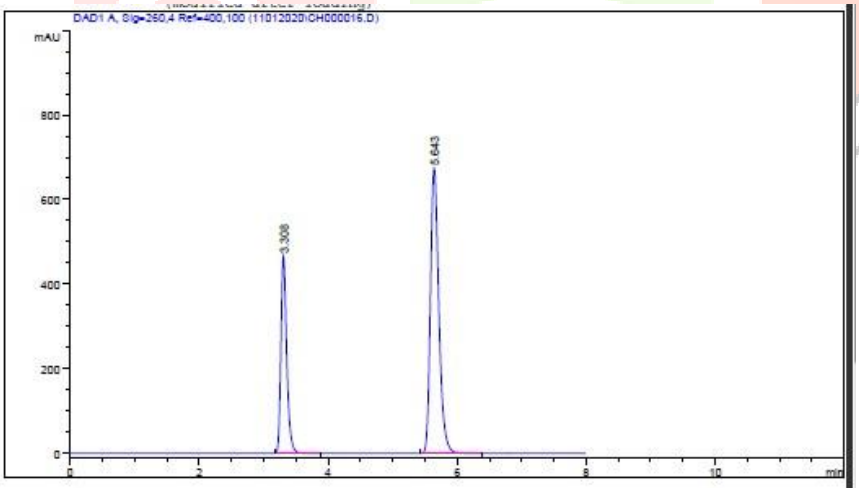
			100%					
Sr no.	µgm/ml	Amt added	Area	Amt found		Amt rcvd		% rcvd

1	10	10	1472.99	19.99		9.99		99.86
2	10	10	1476.2	20.03		10.03		100.31
			Mean	20.01		10.01		100.09
			SD	0.03		0.03		0.32
			%RSD	0.14		0.28		0.32

			120%					
Sr no.	µgm/ml	Amt added	Area	Amt found		Amt rcvd		% rcvd
1	10	12	1622.98	22.05		12.05		100.45
2	10	12	1623.58	22.06		12.06		100.52
			Mean	22.06		12.06		100.49
			SD	0.01		0.01		0.05
			%RSD	0.03		0.06		0.05

Precision :-

The method was established by analyzing various replicate standards of captopril and hydrochlorothiazide .all the solution was analysis thrice in order to record any intra day& inter day variation in the result obtained for intraday in table.



inter day Fig. precision of captopril :

Conc	Area	II	III	Mean	Amt Found	% Amt Fnd	%RSD
40	3066.33	3067.65		3066.99	39.56	0.00	0.96
60	4595.89	4590.61		4593.25	59.71	0.00	3.73
80	6088.47	6096.29		6092.38	79.50	0.00	5.53

chromatogram of precision 40 + 80 MCG 0

Intra day precision of captopril :-

Sr No.	Conc	Area	II	III	Mean	Amt Found	% Amt Fnd	SD	%RSD

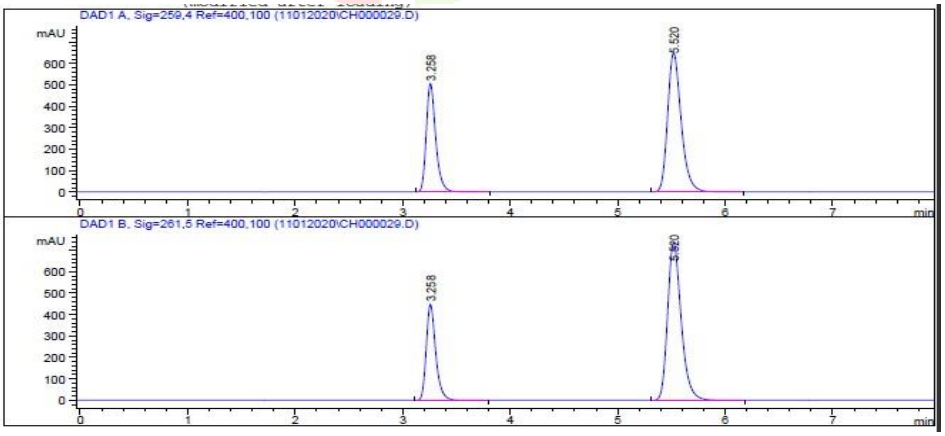
Conc	Area	II	III	Mean	Amt Found	% Amt Fnd	%RSD
40	6123.74	6107.35		6115.55	41.06	102.65	0.96
60	4605.55	4599.04		4602.30	63.01	105.02	4.60
80	3078.36	3081.95		3080.16	83.68	104.60	2.54

Inter day precision of hydrochlorothiazide :-

Sr No.	Conc	Area	II	III	Mean	Amt Found	% Amt Fnd	SD	%RSD
1	20	1461.8	1463.68		1462.74	19.85	99.25	0.96	0.23
2	30	2195.54	2196.23		2195.89	29.95	99.84	0.49	0.02

Robustness:

The Robustness of a system is its capability to remain innocent by small deliberate changes in parameters. To estimate the robustness of the proposed system, small but deliberate variations in the optimized system parameters were done. The effect of changes in mobile phase composition and inflow rate on retention time and trailing factor of medicine peak was studied. The mobile phase composition was changed in ± 1 ml proportion and the inflow rate was varied by ± 0.1 ml/ min of optimized chromatographic condition. The results of robustness studies are shown in.



Robustness of captopril :-

	Change flow								
FLOW RATE-0.6							**FLOW RATE-1.1**		
Sr No.	**Conc**	**µgm/ml**	**Area**			**Sr No.**	**Conc**	**µgm/ml**	**Area**
1		40	210.61			1		40	324.18
2		40	393.94			2		40	316.73
		Mean	302.28					Mean	320.46
		SD	129.63					SD	5.27
		%RSD	42.89					%RSD	1.64
MP			79 + 21 BUFFER			MP	81 + 19 BUFFER		
	Sr No.	Conc	µgm/ml	Area		Sr No.		µgm/ml	Area
	1		40	679.19		1		40	673.22
	2		40	674.26		2		40	677.1
			Mean	676.7				Mean	675.16
			SD	3.49				SD	2.74
			%RSD	0.52				%RSD	0.41
WAVE LENGTH CHANGE			247					249	
	Sr No.	Conc	µgm/ml	Area		Sr No.		µgm/ml	Area
	1		40	189.82		1		40	182.74
	2		40	190.23		2		40	181.47
			Mean	190.0				Mean	182.11
			SD	0.29				SD	0.90
			%RSD	0.15				%RSD	0.49

Robustness of hydrochlorothiazide :-

	Change flow								
FLOW RATE-0.6							**FLOW RATE-0.8**		
Sr No.	**Conc**	**µgm/ml**	**Area**			**Sr No.**	**Conc**	**µgm/ml**	**Area**
1		40	3619.15			1		40	2552.96
2		40	393.94			2		40	2547.09
		Mean	2006.55					Mean	2550.03
		SD	2280.57					SD	4.15
		%RSD	113.66					%RSD	0.16
MP			79 + 21 BUFFER			MP	81 + 19 BUFFER		
	Sr No.	Conc	µgm/ml	Area		Sr No.		µgm/ml	Area
	1		40	679.19		1		40	673.22
	2		40	674.26		2		40	677.1
			Mean	676.7				Mean	675.16
			SD	3.49				SD	2.74
			%RSD	0.52				%RSD	0.41
WAVE LENGTH CHANGE			259					261	
	Sr No.	Conc	µgm/ml	Area		Sr No.		µgm/ml	Area
	1		40	3088.62		1		40	2735.4
	2		40	3091.93		2		40	3199.74
			Mean	3090.3				Mean	2967.57
			SD	2.34				SD	328.34
			%RSD	0.08				%RSD	11.06

Limit of discovery-

The Limit of Discovery(LOD) is the lowest attention of the analyte that gives the measurable response. LOD was calculated used on standard divagation of the response and pitch of the wind.

LOD = 3.3 x Avd. SD/ pitch
= 0.403842 µg/ ml.
Where,
SD = standard divagation
S = pitch
The limit of discovery(LOD) was set up to be 0.403842 µg/ ml.

Limit of quantification(LOQ)-

The quantification limit of an logical procedure is the smallest quantum of analyte in sample, which can be quantitatively determined with suitable perfection and delicacy.

LOQ = 10 x Avd. SD/ pitch
= 1.223762 µg/ ml.

Where,
SD = standard divagation
S = pitch The limit of discovery(LOQ) was set up to be 1.223762 µg/ ml.

Stability study-

Chemical declination presumably represents the most important stability aspect of medicinals.
Pharmaceutical scientists are responsible for examining. the chemical stability of new medicine campaigners, for assessing the impact of stability issues on pharmaceutical development and processing, and for designing strategies to stabilize an unstable emulsion if necessary.
They must understand the kinetics of chemical declination, both in result and in the solid state.
They must also understand the generally encountered declination pathways of active pharmaceutical constituents(APIs), as well as practical approaches for performing declination studies.

Brand Name Tab
Result Preparation
Brand Name

Total weight of 20 tab
Greasepaint wt. = 16.24 gms
AvgPowder Weight = 0.812 gms.
Tab for 15 mg = 20 x812/ 400 = 40.6 mg 1)
Take 40.6 mgs in 10 ml MeOH. i. e = 1000 µgm/ ml HCZ & 2000 µg/ ml CAPT.
(Sonicate 30 min also fitter with 0.45 µm),------- STOCK1I

Tab Assay- Take 40 20 µgm/ ml for assay 0.2 ml FROM STOCK II announcement MAKEUP VOL 10ML WITH m. phase.

Discussion

Attempts were made to develop RP- HPLC system for contemporaneous estimation Captopril and Hydrochlorothiazide from Captopril- H tablet.
For the RP- HPLC system, grade System UV Sensor and C18 column with 100 mm x 4.6 mm. and 2.5 µm flyspeck size.
Methanol Water(4060) pH 3 with OPA was used as the mobile phase for the system.
The discovery wavelength was 261 nm and inflow rate was 0.7 ml/ min. In the advanced system, the retention time of Captopril and Hydrochlorothiazide were set up to be 1.78 min and 3.10 min. The developed system was validated according to the ICH guidelines.
The linearity, perfection, LOD, LOQ, range, robustness were within the limits as specified by the ICH guidelines. Hence the system was set up to be simple, accurate, precise, profitable and reproducible.
So the proposed styles can be used for the routine quality control analysis of Captopril and Hydrochlorothiazide.
The stability study was set up to be 1.3191 DEG – 01. in bulk medicine as well as in phrasings.

Summary :-
A combination of captopril and hydrochlorothiazide is clinically used in combination in the treatment of anti hypertensive drug .
The present work deal with the contemporaneous estimation of captopril and hydrochlorothiazide in bulk dosage form by RP- HPLC technique .

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VII . Reference-

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5) Vibha Gupta, Ajay Deep Kumar Jain, N. S. Gill, Kapil Gupta. Development and confirmation of HPLC system- A review. Int. Res. J. Pharma. App. Sci. 2012; 2(4) 17- 25.

6) Malviya R, Bansal V, and confidante O.P. and Sharma P.K. High Performance Liquid Chromatography A Short Review. J. Glo. Pharma. Tech. 2010; 2(5) 22- 26.

7) Phani.R.S.Ch, K.R.S. Prasad and Useni Reddy Mallu. Scientific 12) Approach for RP- HPLC Method Development Complete Review. 13) Int. J. Sci. Inno. Slice. 2012; 2(6) 218- 228.

8) D. Zhou1, W.R. Porter2 and G.G.Z. Zhang1 1AbbVie Inc., North Chicago, IL, United States 2Abbott Laboratories, Abbott Park, IL, United States

9) Tabata T, Makino T, Kashihara T, Hirai S, Kitamori N, Toguch H. Stabilization of a new antiulcer medicine(lansoprazole) in the solid lozenge forms. Drug Dev Ind Pharm 1992; 181437_47.

10) Tabata T, Makino T, Kashihara T, Hirai S, Kitamori N, Toguchi H. Stabilization of a new antiulcer medicine(lansoprazole) in th solid lozenge forms. Drug Dev Ind Pharm 1992; 181437_47.