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SIMULTANEOUS DETERMINATION AND VALIDATION OF HYDROCHLORTHIAZIDE & CANDESARTAN IN BULK DRUG AND MARKETED FORMULATION BY RP-HPLC

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ABSTRACT

The aim of this work is to develop simple and precise Reverse phase High performance liquid chromatography (RP-HPLC) method for simultaneous determination of Hydrochlorthiazide & candesartan using single method in bulk drug and marketed formulation. Hydrochlorothiazide in various solvents, it was found 50 Mm KH_2PO_4: Acetonitrile (30:70) best suitable mobile phase. The selection of separation variable like mobile phase composition, flow rate and detection wavelength. The study shows the system suitability parameter less S.D. value shows good result and the result of linearity of Candesartan it was found 3-6 µg/ml and the standard calibration curve of Candesartan regression equation was determined and R^2 was calculated and it was found to be 0.999 so it is linear between the concentration range 8-40 µg/ml. The study shows the result of linearity of Hydrochlorothiazide it was found 5-25 µg/ml.

KEYWORDS: Hydrochlorthiazide, Candesartan, RP-HPLC, Acetonitrile, Chromatography, Linearity.

1 INTRODUCTION

One of the early problems with liquid chromatography was the slow rate at which the analysis took place. Early methods used gravity feed, and it was not uncommon for an analysis to take several days to complete. This led to great delay. Also the excessive time on the column inevitably led to loss of resolution by diffusion, and so on. Consequently, for a number of years liquid chromatography was not widely used as means of separating organic compounds. This problem was largely overcome by the advent of high performance liquid

chromatography (HPLC). In this system pressure is applied to the column, forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC. Emphasis has been placed on the size of the particles making up the substrate. It has been found that the smaller size, better the resolution. Pressures used normally range from 30 to 200 atm, depending on the type of column used. The pressure is varied to provide the optimum linear flow rate of the mobile phase. It is that pressure which gives the smallest theoretical plate.

CANDESARTAN (2-ethoxy-1-({4-[2-(2*H*-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1*H*-1,3-benzodiazole-6-carboxylic acid)-Candesartan is an angiotensin-receptor blocker (ARB) that may be used alone or with other agents to treat hypertension. It is administered orally as the prodrug, candesartan cilexetil, which is rapidly converted to its active metabolite, candesartan, during absorption in the gastrointestinal tract. Candesartan lowers blood pressure by antagonizing the renin-angiotensin-aldosterone system (RAAS); it competes with angiotensin II for binding to the type-1 angiotensin II receptor (AT1) subtype and prevents the blood pressure increasing effects of angiotensin II.

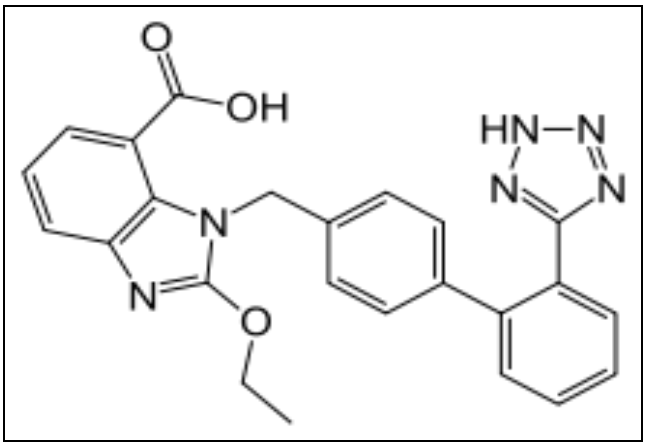

Fig. 1: Molecular structure of Candesartan.

HYDROCHLOROTHIAZIDE

(6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide)

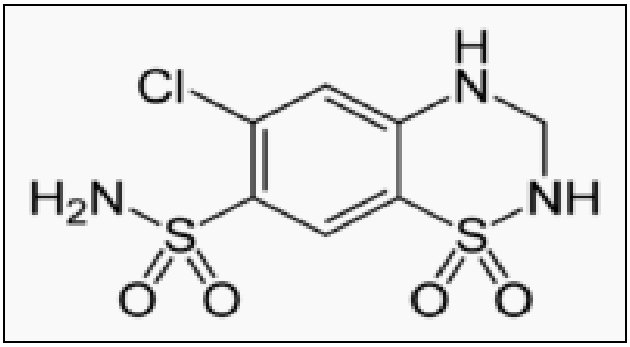

Fig. 1: Molecular structure of Hydrochlorothioazide.

Hydrochlorothiazide, abbreviated HCTZ, HCT, or HZT, is a diuretic drug of the thiazide class that acts by inhibiting the kidneys' ability to retain water. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance.[2] Hydrochlorothiazide is a calcium-sparing diuretic, meaning it can help the body get rid of excess water while still keeping calcium.

2 EXPERIMENTAL WORK

2.1 MATERIALS

Table No. 1. Chemicals and Solvents Used.

S. No.	Chemicals	Manufacturer
1	Candesartan	Macleod's Pharmaceutical Ltd.
2	Hydrochlorothiazide	Kalindi Medicure Pvt. Ltd.
3	Acetonitrile (HPLC)	Merck Ltd., India
4	Methanol (HPLC)	Merck Ltd., India
5	Water (HPLC)	Merck Ltd., India

2.2 INSTRUMENTS

- Shimadzu HPLC with UV Detector (LC2010A)
- Chemito 2600 Double beam UV-Vis spectrophotometer
- Phennomenax C18 (25x 4.6 mm)
- Pump(Analytical 2010)
- Precision loop injector (Rheodyne 20µl)
- Electronic Balance (AX205)
- pH Meter (101E)
- Sonicator (Life care)

2.3 METHODS

2.3 Analytical Method Development of Candesartan and Hydrochlorothiazide By Hplc

2.3.1- Mobile Phase Selection

Initially to estimate Candesartan and Hydrochlorothiazide number of mobile phase in different ratio were tried. Results are shown in (Table no 03.). Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was 50 mM KH_2PO_4 (pH4.0 with OPA): Acetonitrile in the ratio of 30**:**70 v/v. The mobile phase was filtered through 0.45□ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

6.3.8- Analysis of Tablet Formulation

6.3.8.1- Preparation of Mixed Standard

The commercial formulations of Candesartan and Hydrochlorothiazide are in the ratio of 32:12.5 Based on this fact six mixed standards were selected for quantitative analysis, which gave satisfactory results. Stock solution was prepared in the same manner. Further dilutions were made to prepare the mixed standard of desired concentration.

6.3.8.2- Assay of tablet formulation

For analysis of the formulation, twenty tablets taken and determine the average weight. Powder equivalent to weight 32 mg of Candesartan was transferred to 10 ml volumetric flask and dissolved in HPLC grade methanol. The solution was shaking vigorously for 10 mins and filtered through Whatman filter paper no.41, then volume was made up to mark with methanol. From the above solution 1 ml of solution was taken and diluted to 10 ml with mobile phase to get a solution containing 100 µg/ml. From the above solution 3.2 ml of solution was taken and diluted to 10 ml with mobile phase to get a solution containing 32 µg/ml. of Candesartan and corresponding concentration of Hydrochlorothiazide 12.5 µg/mL. The solution contains Candesartan and Hydrochlorothiazide in the proportions of 32:12.5. The amounts of Candesartan and Hydrochlorothiazide calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with tablet formulation. Result is shown in (Table no.09).

3. RESULT AND DISCUSSION

3.1 Identification

FTIR spectrum of Candesartan and Hydrochlorothiazide

FTIR spectrum- IR absorption spectra Candesartan and Hydrochlorothiazide was obtained by KBr pellet method. Spectra of pure Candesartan and Hydrochlorothiazide shown in the Figure 3 and Figure 4.

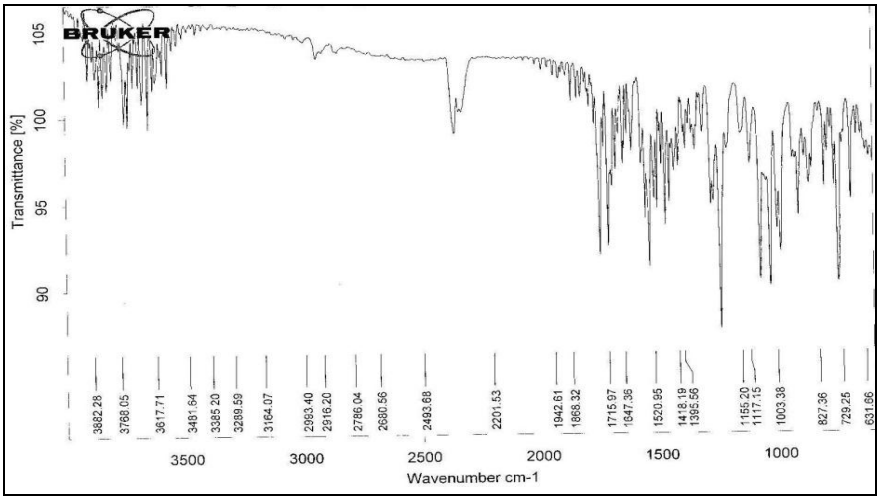

Fig. 3 IR Spectra of Candesartan.

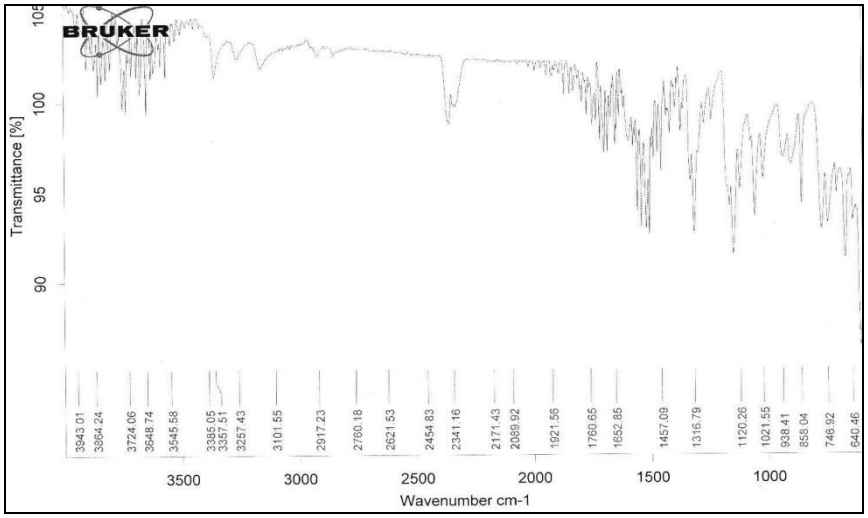

Fig. 3 IR Spectra of Hydrochlorothiazide

3.2- Mobile Phase Selection

Table No. 2 - Mobile Phase selection.

Mobile phase	Ratio	Flow rate	Retention time		Conclusion
			CANDE	HCZ	
Methanol	100	1.00 ml/min.	11.67	Not Found	Not Suitable
Methanol: water	50:50	1.00 ml/min.	8.56	Tailing	Not Suitable
Acetonitrile : Methanol	60 : 40	1.00 ml/min	Not found	Tailing	Not Suitable
50 Mm KH_2PO_4: ACN	50:50	1.00 ml/min	7.216	Tailing	Not Suitable
50 Mm KH_2PO_4(pH4 with OPA): ACN	30:70	1.00 ml/min	8.701	5.471	Most Suitable

Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was 50 mM KH_2PO_4(pH4 with OPA): Acetonitrile in the ratio of (30:70 v/v). The mobile phase was filtered through 0.45□ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

3.3 -System Suitability Parameters

Table No. 3- Result of System Suitability Parameters for Candesartan.

System suitability Parameter →	RT	AUC	Theoretical plates	Tailing factor
Rep-1	8.701±0.2 min	5214.365	2558	1.22
Rep-2	8.700±0.3 min	5245.145	2585	1.24
Rep-3	8.702±0.4 min	5241.363	2581	1.23
Mean	8.701±0.3min	5233.624	2574	1.20
S.D.	8.0008±0.3min	13.70565	1.45	0.015

Table No. 4: Result of System Suitability Parameters for Hydrochlorothiazide.

System suitability Parameter →	RT	AUC	Theoretical plates	Tailing factor
Rep-1	5.470±0.4min	6574.236	2590	1.10
Rep-2	5.471±0.3min	6547.236	2592	1.12
Rep-3	5.472±0.2min	6589.236	2590	1.11
Mean	5.470±0.3min	6570.236	2591	1.10
S.D.	8.0008±0.3min	17.37815	1.45	0.01

3.4 -Linearity and Calibration Graph

Table No. 5 - Result of Linearity of Candesartan.

Std. Conc. □g/ml	0	8	16	24	32	40
1	0	5214.365	10454.236	14689.125	20578.365	25455.236
2	0	5245.145	10467.336	14685.256	20547.235	25469.365
3	0	5241.363	10468.225	14701.365	20569.325	25471.236
Mean	0	5233.624	10463.27	14691.92	20564.98	25465.28
SD	0.00	13.70565	6.395245	6.866073	13.07571	7.142434
%RSD	0.000	0.261877	0.061121	0.046734	0.063582	0.028048

Table No.6: Result of Analysis for Candesartan and Hydrochlorothiazide in Tablet Formulation.

Std Conc. µg/ml	Candesartan	Hydrochlorothiazide
	32	12.5
Rep-1	31.99	12.5
Rep-2	31.99	12.45
Rep-3	32.00	12.48
% found *		

Rep-1	99.96875	100
Rep-2	100	99.6
Rep-3	100.0313	100.241
Mean	100	99.94699
SD	0.031255	0.323754
% RSD	0.031255	0.323925

***Each reading is mean reading of three batch of formulation**

Table No. 7: Response Ratio Data for Linearity of Candesartan.

Replicates	Concentration (□g/ml)	Mean AUC	Response Ratio
Rep-1	8	5233.624	654.203
Rep-2	16	10463.27	653.9544
Rep-3	24	14691.92	612.1633
Rep-4	32	20564.98	642.6556
Rep-5	40	25465.28	636.632
Mean		639.9217	
S.D.		17.24599	
R.S.D.		2.695017	

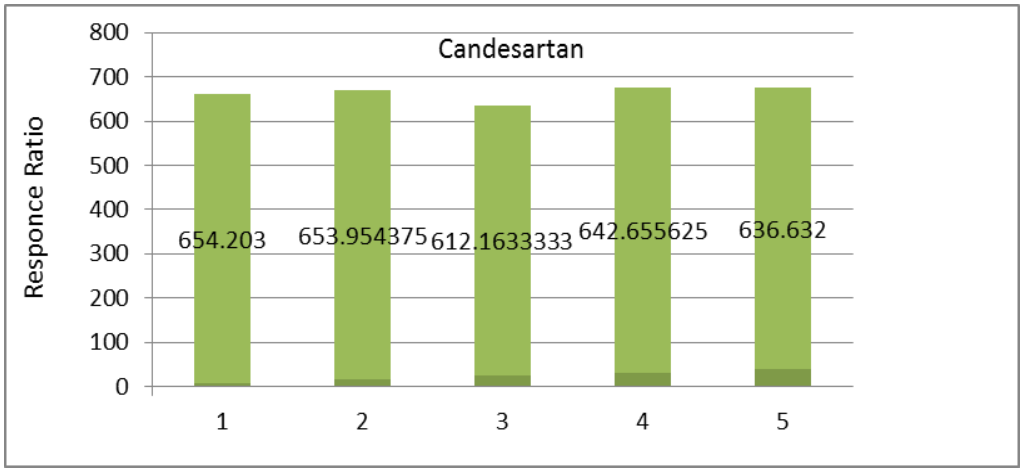

Fig. 4: Response Ration graph for Linearity of Candesartan.

Table No. 8: Response Ratio Data for Linearity of Hydrochlorothiazide.

Replicates	Concentration (□g/ml)	Mean AUC	Response Ration
Rep-1	5	3270.279	654.0558
Rep-2	10	6570.236	657.0236
Rep-3	15	9257.464	617.1643
Rep-4	20	12450.7	622.535
Rep-5	25	15502.46	620.0984
Mean		634.1754	
S.D.		19.62338	
R.S.D.		3.094315	

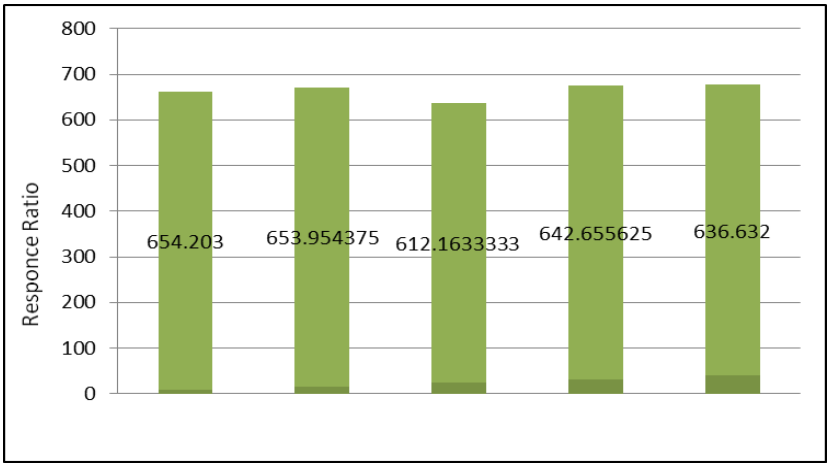

Fig. 5: Response Ration graph for Linearity of Hydrochlorothiazide

3.5. -Result of Accuarcy

Table No. 9- Recovery Studies of Formulation.

Level of Recovery (%)	80		100		120	
	CANDE	HCZ	CANDE	HCZ	CANDE	HCZ
Amount present (mg)	10 10 10	10 10 10	10 10 10	10 10 10	10 10 10	10 10 10
Amount of Std. added (mg)	8 8 8	10 10 10	12 12 12	8 8 8	10 10 10	12 12 12
Amount recovered (mg)	8.10 7.95 8.00	10.00 10.01 9.90	12.0 12.02 11.98	7.95 7.96 8.00	9.98 10.02 9.99	11.98 12.00 11.97
% Recovery	101.25 99.375 100.00	100.00 100.10 99.00	100 100.16 99.83	99.37 99.50 100.00	99.8 100.2 99.9	99.83 100.00 99.75

Table No. 10: Statistical Validation of Recovery Studies.

Level of Recovery (%)	Drug	% Recovery	Standard Deviation*	% RSD
80	CANDE	100.28	0.955	0.953
	HCZ	99.7	0.608	0.61
100	CANDE	100	0.167	0.167
	HCZ	99.625	0.331	0.332
120	CANDE	99.967	0.208	0.208
	HCZ	99.861	0.127	0.127

***Denotes average of three determinations.**

3.5- Result of Precision

(A) Repeatability

Table No. 11- Results of analysis Data of Tablet Formulation.

Drug	Label claim Mg/ml	Amount found* mg/ml	Label claim (%)	S.D.	% RSD
CANDE	32	31.99	99.97	0.155	0.159
HCZ	12.5	12.48	99.84	0.154	0.156

(B) Intermediate Precision- (Inter-day and Intra-day Precision):-

Table No. 12: Intra-day and Inter-day Precision.

Intra-day Precision			Inter-day Precision		
	% Label Claim			% Label Claim	
	CANDE	HCZ		CANDE	HCZ
After 1hr	99.90	98.90	First day	97.00	96.00
After 2hr	99.45	98.50	Second day	96.50	95.50
After 3hr	99.10	98.10	Third day	95.00	94.50
After 4hr	99.00	98.00			
After 5hr	98.95	97.80			
After 6hr	98.80	97.50			
Mean	99.2	98.13	**Mean**	96.16	95.33
SD	0.37	0.45	**SD**	0.84	0.62
% RSD	0.37	0.46	**% RSD**	0.88	0.65

(C) Analyst to Analyst

Table No. 13-Analyst to Analyst.

Analyst	Label claim mg/ml		Amount found* mg/ml		Label claim (%)		S.D.		% RSD	
	CANDE	HCZ	CANDE	HCZ	CANDE	HCZ	CANDE	HCZ	CANDE	HCZ
1	32	12.5	31.95	12.45	99.84	99.60	0.112	0.098	0.110	0.115
2	32	12.5	31.99	12.48	99.97	99.84	0.212	0.211	0.225	0.220

3.6. -Result of Robustness

Table No. 14 Result of Robustness of Formulation.

Compound	% RSD in Normal	Changed Condition n= 6	
Temperature		- 5 ^{o}C	+ 5 ^{o}C
CANDE	0.52	0.68	0.27
HCZ	0.51	0.56	0.23
Flow rate		(-10%)	(+10%)
CANDE	0.45	0.56	0.65
HCZ	0.33	0.52	0.54
Mobile phase ratio		- 2 %	+ 2 %
CANDE	0.34	0.89	0.54
HCZ	0.54	0.67	0.65

4 SUMMARY AND CONCLUSION

Table no. 02. Shows the selection of mobile phase of Candesartan and Hydrochlorothiazide in various solvents, it was found 50 Mm KH_2PO_4: Acetonitrile (30:70) best suitable mobile phase. Table no. 04. Shows the selection of separation variable like mobile phase composition, flow rate and detection wavelength. Table no. 03, 04. Shows the result of system suitability parameter less S.D. value shows good result. Table no. 05. shows the result of linearity of Candesartan it was found 3-6 µg/ml. Fig. 05 shows the standard calibration curve of Candesartan regression equation was determined and R^2 was calculated and it was found to be 0.999 so it is linear between the concentration range 8-40 µg/ml. Table no. 06 shows the result of linearity of Hydrochlorothiazide it was found 5-25 µg/ml. Fig. 06. shows the standard calibration curve of Hydrochlorothiazide regression equation was determined and R^2 was calculated and it was found to be 0.999 so it is linear between the concentration range 5-25 µg/ml. Fig. 07 and 08 shows the chromatogram of Candesartan and Hydrochlorothiazide respectively in the optimized mobile phase. Table no. 09 shows the result of analysis of Marketed Tablet formulation by HPLC method, the RSD of the average of 6 determinations was found to be below 1. So that method can be used for the estimation of Candesartan and Hydrochlorothiazide simultaneously from its Tablet dosage form. Table no. 12, 13. shows the Recovery results of the two different formulations on addition of Standard drug by HPLC. The Mean percentage Recovery and RSD were found to be less than 2 so the method was found to be more accurate without any interference.

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