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Estimation Of Captopril And Hydrochlorothiazide In Bulk And Pharmaceutical Dosage Form Using Ultra Performance Liquid Chromatography With Photodiode Array Detector

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Abstract: Quick and easy, specific, isocratic technique was created and tested for simultaneous estimation of Captopril and Hydrochlorothiazide (HCTZ) using Waters Acquity UPLC. The separation was accomplished using C18 column (100 × 2.1 mm, 1.7 µ) and a mobile phase of acetonitrile and 0.1% formic acid (60:40% v/v) with a flow of 0.2 mL/min at 210 nm using PDA detector. The retention times of Captopril and Hydrochlorothiazide were 0.772 min, 1.679 min. Five minutes run time was used to separate drugs. Validation of the proposed method was carried out according to ICH guidelines. The linearity range was observed from 5 - 30 µg/mL each of Captopril and HCTZ. A stress study was conducted for the sample solution with acid, alkali, heat, peroxide, light and the percentage degradation was calculated.

Keywords: Captopril, HCTZ, isocratic, ultra-precision liquid chromatography, photo diode array detector.

1. INTRODUCTION

1.1Profile of the drugs

Captopril, also known as Capoten, is an ACE inhibitor [1] used to treat high blood pressure[2-3] (hypertension) and some forms of congestive heart failure[4] (CHF). The first oral ACE inhibitor discovered for hypertension therapy was captopril. Unlike beta-blockers [5-6] it does not make you feel tired[7]. Most ACE Inhibitors have the unwanted side effect of inducing hyperkalemia[8-9], hence a diuretic is often prescribed along with the medicine. Vasodilation[10] and inhibition of certain renal function activities are the basis for captopril's primary clinical use. Hypertension, cardiac diseases such congestive heart failure and following myocardial infarction[11-12] and preservation of kidney function in diabetic nephropathy [13] are prime examples of the settings in which these advantages become most apparent. In addition, research suggests that it may have mood-boosting effects for certain people. This finding is in line with the fact that animal screening models have shown that this drug has potential antidepressant[14-15] action, despite the fact that one research has shown no such effect. No published randomized controlled trials including people with depression have been found. The possibility of using it to treat cancer [16] has also been explored. Some metallo—lactamases [17-18] were also observed to be inhibited by the stereoisomers of captopril [19].

Hydrochlorothiazide is a diuretic often used to treat hypertension and fluid retention. In addition, it may be used to lower the incidence of kidney stones in those whose urine already has a high calcium concentration and to treat diabetes insipidus and renal tubular acidosis [20-21]. When compared to chlortalidone[22], hydrochlorothiazide is not as effective in reducing the risk of a heart attack or stroke[23-24]. Hydrochlorothiazide is beneficial when used in conjunction with other blood pressure drugs. Electrolyte imbalances [25-26] are also possible, with low blood potassium and, less often, low blood sodium, gout, high blood sugar, and dizziness upon standing among the list of potential adverse effects. Although it has been suggested that those who are allergic to sulfa medications are also more likely to be allergic to hydrochlorothiazide, there is no evidence to

support this. Although it is safe for use during pregnancy, it is not often considered a first-choice treatment for pregnant women. As a thiazide, it helps the kidneys excrete more water than they take in. At first, this lowers blood volume, which in turn affects cardiac output. Scientists think it can reduce peripheral vascular resistance over time. So, an attempt was made to construct a basic, isocratic, selective, ultra performance liquid chromatographic method and validate the developed method. Chemical structures of Captopril and HCTZ were shown in **figure 1.**

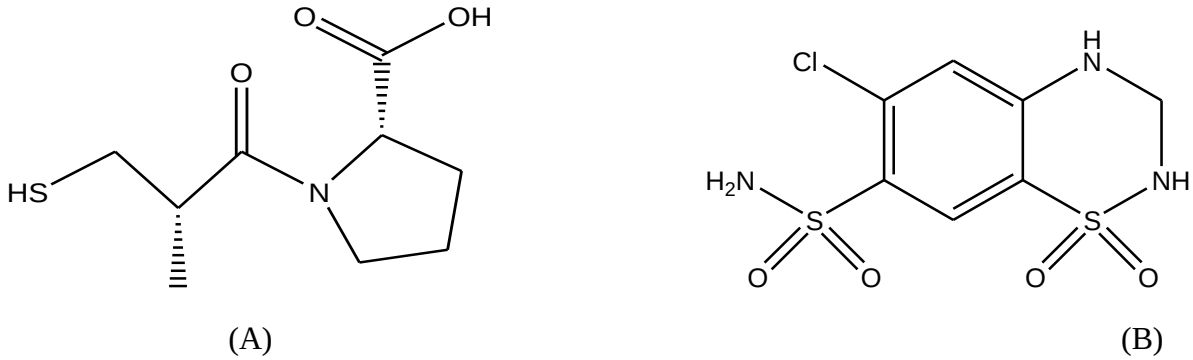

Fig 1: Chemical structure for (A) Captopril and (B) Hydrochlorothiazide

1.2 Literature Survey

In the past few years, there are no reports about using UPLC to measure Captopril and Hydrochlorothiazide[27-29]. In this method, we tried to come up with a selective, reliable, and new way to measure Captopril and Hydrochlorothiazide using Ultra Performance Liquid Chromatography (RP-HPLC).

1.3 Aim and objectives of the Present Investigation

In the present investigation, we quantify the Captopril and Hydrochlorothiazide in Tablets by Stability-Indicating Ultra Performance Liquid Chromatography. The separation was accomplished using C18 column (100 × 2.1 mm, 1.7 µ) and a mobile phase of acetonitrile and 0.1% formic acid (60:40% v/v) with a flow of 0.2 mL/min at 210 nm using PDA detector. The retention times of Captopril and Hydrochlorothiazide were 0.772 min, 1.679 min. Five minutes run time was used to separate drugs.

II.RESEARCH METHODOLOGY

2.1Reagents and Chemicals

HPLC grade solvents were used in this study**.** 99.9% pure Formic acid (Merck), acetonitrile (HPLC grade) and HPLC water were used. Captopril and HCTZ pure drugs (99.9 % purity) and tablets were procured from Glenmark Pharmaceuticals Limited, Mumbai, India.

2.2 Instrumentation

Waters Acquity type UPLC with quaternary pump, PDA detector and empower 2.0 software was used for this study.

2.3 Analytical Method development

Many trials were conducted to develop a UPLC method for Captopril and HCTZ using different columns, flow rates, mobile phases and diluents and finally an isocratic method was developed. The method was optimized C_{18} column (100 x 2.1 mm, 1.7µ), using acetonitrile and 0.1 % formic acid (60:40 v/v) as mobile phase with a flow of 0.2 mL/min at 210 nm. All the separations were carried out for a run time of 5 min and a good symmetrical peak was obtained at a retention times of Captopril and HCTZ were at 0.772 min. and 1.679 min respectively. **Table 1** shows the details the ideal chromatographic conditions.

Table.1: Results of Method Optimization**.**

Parameter	Value
Column	C_{18} column (100 × 2.1 mm, 1.7 µ)
Changeable Phase	ACN: 0.1 % v/v formic acid (60: 40 % v/v)
Mode of elution	Isocratic
rate of flow	0.2 mL /min.
Detected Wavelength	210 nm
Volume of injection	5 µL
Run time	5 min.
Captopril retention time HCTZ retention time	0.772 min. 1.679 min

2.4 Method Validation

The method was validated according to the guidelines of ICH Q2 (R1). The method was validated for parameters like linearity, LOD & LOQ, specificity, precision (inter and intra - day), robustness, accuracy, and system suitability parameters.

2.4.1System Suitability

Parameters for device appropriateness were determined, including theoretical plate number, duration, peak area, tailing factor and resolution. **2.4.2 Linearity and Accuracy**

The proportionality of peak area and concentration (µg/mL) were determined by calibration curve using standard solutions (Captopril and HCTZ) of 5 – 30 µg /mL (5, 10, 15, 20, 25, 30 µg/mL) from which correlation coefficient, slope and y-intercept were calculated. The percentage recovery was calculated at three levels (50 %, 100 %, and 150 %) by the standard addition method. Three solutions were prepared at each level, injected in 3 replicates and analyzed for % RSD.

2.4.3 Precision

The accuracy of the procedure was evaluated while comparing disparities in intraday and intermediate precision.

Within-day analyses included find out by analyzing the sample solution of Captopril and HCTZ six times on the identical day with just like that original conditions. Considering the accuracy of the system was studied same lab by analyzing the data using variant examiners and instruments. The approach is extremely accurate, with percentage RSD values of less than 2%. Good drug recoveries were attained at each added concentration, suggesting that the procedure was precise.

2.4.4 Robustness

Deliberate modifications of chromatographic parameters like rate of flow (± 10%), composition of the mobile phase and (± 10 %) were done to monitor the changes in maximum intensity, retention period, theoretical plates, RSD (Relative Standard Deviation) in Percentage).

2.4.5 LOD and LOQ

Lowest quantifiable analyte load detected and minimum quantifiable analyte concentrations were calculated using the provided formulae:

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

Where σ denotes the variance from the linearity curve

S denotes sloping calibration curve

2.4.6 Forced Degradation

The stable nature of the drug in sample was tested under various conditions by subjecting the sample solution to treatments like acid, alkali, peroxide, heat and light for the degradants in the sample and the percentage degradation was calculated. A fresh stock of 200 µg/mL each of Captopril and HCTZ was prepared to carry out forced degradation study.

2.4.6.1Acid Degradation

A 1 mL from the stock (200 µg/mL) was poured into a 10 mL volumetric flask and add 1 ml 1N HCl, heated at 60˚C for 60 min., cooled, neutralized with 1N NaOH and made up with diluent.

2.4.6.2 Alkali Degradation

From the stock (200 µg/mL), 1 mL was placed in a 10 mL volumetric flask and add 1 mL 1N NaOH, heated at 60˚C for 60 min., cooled, made up with the diluent after neutralization with 1N HCl.

2.4.6.3 Peroxide Degradation

1 mL of the stock solution was transferred to volumetric flask of ten millilitres and adds 1 mL of 3% v/v hydrogen peroxide, heated at 60˚C for 60 min., cooled and made up with diluent.

2.4.6.4 Thermal Degradation

A one mL of the stock solution was transferred to a 10 mL volumetric flask, heated to 80˚C for 60 min. After that cooled and made up with the diluent.

2.4.6.5 Photolytic degradation

One mL of the stock solution was transferred to a 10 mL volumetric flask, kept in UV chamber at 210 nm for 60 min., removed and made up with the diluent.

III.RESULTS AND DISCUSSION

To develop a UPLC method for Captopril and HCTZ using different columns, flow rates, mobile phases and diluents and finally an isocratic method was developed. The method was optimized C_{18} column (100 x 2.1 mm, 1.7µ), using acetonitrile and 0.1 % formic acid (60:40 v/v) as mobile phase with a flow of 0.2 mL/min at 210 nm. All the separations were carried out for a run time of 5 min and a good symmetrical peak was obtained at a retention times of Captopril and HCTZ were at 0.772 min. and 1.679 min respectively. A rapid, reliable, and responsive HPLC technique has been developed. When the system's efficiency-influencing factors were fine-tuned, the resulting strategy showed excellent sensitivity and selectivity. There is no HPLC method mentioned in the literature. Designing an UPLC approach for the in vitro quantification of combination medications is therefore intriguing. According to the ICH stability requirements, a number of forced conditions, including heat, alkali, acidic, oxidative, UV, and reductive, have been researched. The process was validated and the results fell within the permitted range as per ICH standards.

3.1 System Suitability

A retention times of Captopril and HCTZ were 0.772 min. and 1.679 min, theoretical plates > 5000, tailing factor < 1.5 were achieved for the developed method. **Fig 2** shows the standard chromatogram.

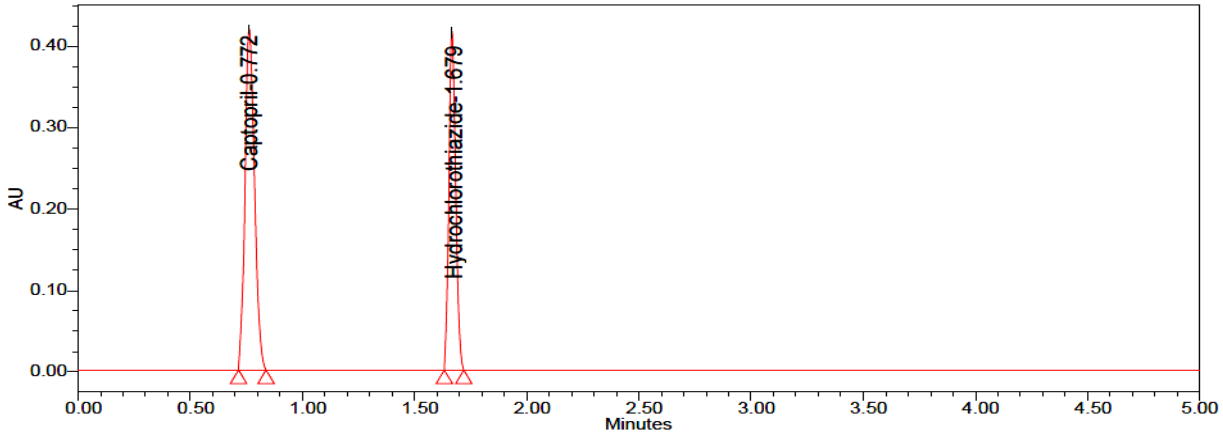

Fig.2: Standard Chromatogram of UPLC

3.2 Specificity

There were no interferences due to excipients, solvents etc. during the sample retention time. Hence the developed method was specific (**Fig 3.**).

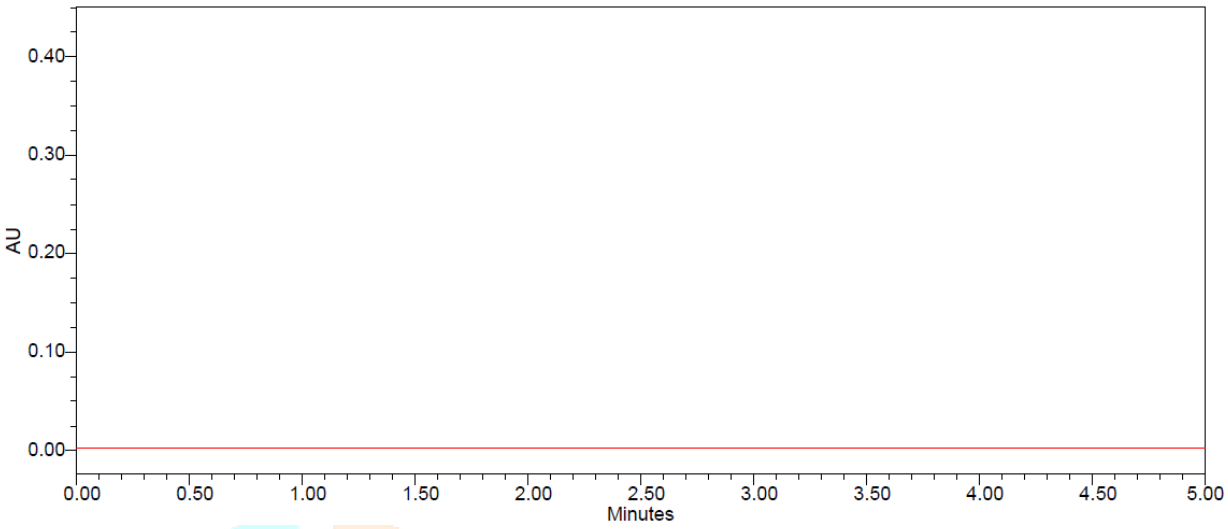

Fig. 3: UPLC blank chromatogram

3.3 Linearity

The dilutions of 5 - 30 µg/mL (5, 10, 15, 20, 25, 30 µg/mL) were made from the reference stock and analyzed they represented in **fig 4**. A calibration curve was plotted by regression analysis for concentration (µg/mL) vs peak area **fig 5.** Represents the calibration plots of Captopril and HCTZ and correlation coefficient, slope, intercept were calculated as mentioned in **table 2**.

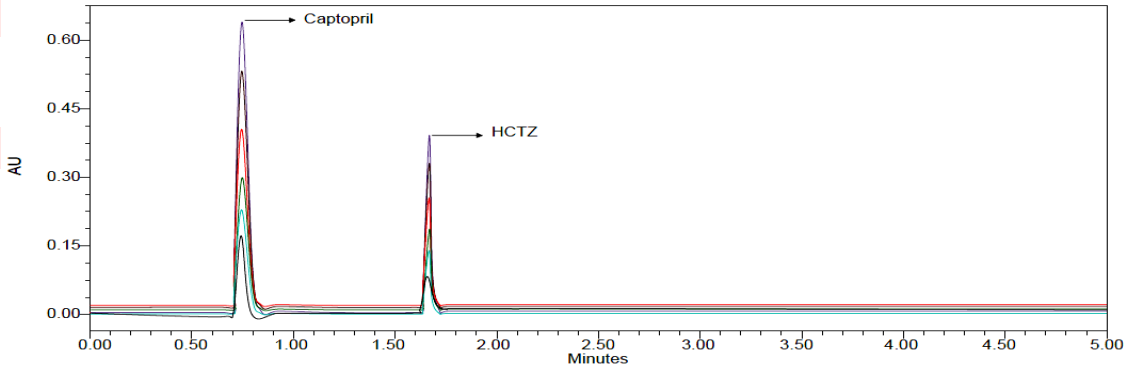

Fig 4: Overlay Chromatogram of Linearity

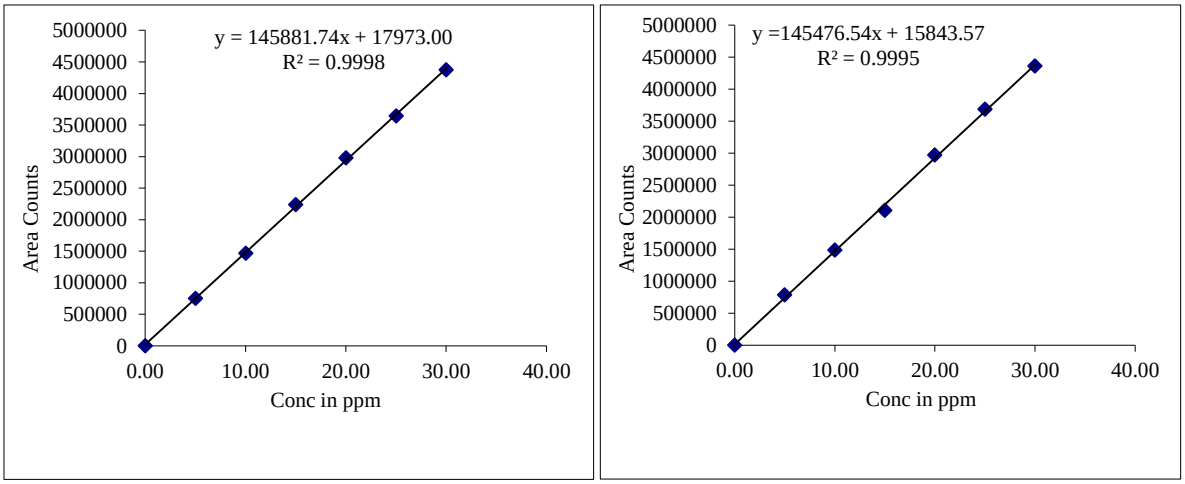

Fig 5: Calibrating curve for (A) Captopril and (B) HCTZ

Table.2: HPLC results of Linearity

S. No	Captopril		HCTZ	
	Conc. (µg/mL)	Number of areas	Conc. (µg/mL)	Number of areas
1	5.00	748859	5.00	784301
2	10.00	1463289	10.00	1482965
3	15.00	2236510	15.00	2105299
4	20.00	2977973	20.00	2968191
5	25.00	3643811	25.00	3685466
6	30.00	4372952	30.00	4359720
the correlation coefficient		0.99986		0.99953
Slope		145881.74		145476.54
intercept		17973.00		15843.57

3.4 Accuracy

Exactness was done by standard strategy for adding at 3 degree (50, 100) %, 150 %) both the percentage recovery was calculated as provided data in **table 3**.

Table.3: Accuracy results of (A) Captopril and (B) HCTZ

A

Level (%)	Sample peak area	Amount of standard added (µg/mL)	Conc. (µg/mL)	% Recovery ± SD, %RSD (n = 3)
50	1473139	10	9.92	99.9 ± 0.65, 0.65
	1493221	10	10.05	
	1483867	10	9.99	
100	2994035	20	20.16	100.4 ± 0.51, 0.50
	2983103	20	20.09	
	2964367	20	19.96	
150	4445321	30	29.93	99.4 ± 0.46, 0.46
	4405234	30	29.66	
	4432157	30	29.84	

B

Level (%)	Sample peak area	Amount of standard added (µg/mL)	Conc. (µg/mL)	% Recovery ± SD, %RSD (n = 3)
50	1459441	10	9.91	99.5 ± 0.33, 0.33
	1468234	10	9.97	
	1467126	10	9.962	
100	2928921	20	19.888	100.0 ± 0.66, 0.66
	2966023	20	20.14	
	2938308	20	19.952	
150	4380308	30	29.743	99.3 ± 0.14, 0.15
	4389124	30	29.803	
	4392564	30	29.827	

% percentage, Micrograms Per Milliliter, Conc. concentration, % w/w percentage percentage, number of tests, standard deviation Dispersion (in %), SD, and RSD percentage Standard Deviation, Relative

3.5 Precision

The % RSD calculated for inter - day and intra - day were precise and repeatable as mentioned in **table 4** respectively.

Table 4: Results of (A) Inter-day precision and (B) Intra-day precision

A

S.No	Captopril				HCTZ			
	Day-1 Area counts	**Day-1 % assay**	**Day-2 Area counts**	**Day-2 % assay**	**Day-1 Area counts**	**Day-1 % assay**	**Day-2 Area counts**	**Day-2 % assay**
1	2991234	100.7	2945124	99.1	2959786	100.5	2966320	100.7
2	2967328	99.9	2985465	100.5	2962848	100.6	2941532	99.9
3	2971384	100.0	2952133	99.4	2971024	100.9	2978564	101.2
4	2919784	98.3	2912605	98.0	2957471	100.4	2956352	100.4
5	2953128	99.4	2954366	99.4	2938357	99.8	2986953	101.4
6	2933354	98.7	2974581	100.1	2921687	99.2	2974815	101.0

B

S.No	Captopril			HCTZ		
	Conc. (µg/ml)	Area	percent assay	Conc. (µg/ml)	Area	percent assay
1	20	2969274	100	20	2948679	100.1
2		2999553	101		2940432	99.8
3		2994189	100.8		2953387	100.3
4		2985510	100.5		2969317	100.8
5		2964115	99.8		2970667	100.9
6		2983067	100.4		2958679	100.5

3.6 Limit of detection and Limit of quantification

The calculated values of LOD and LOQ for Captopril were 0.06 and 0.2 µg/mL and for HCTZ were 0.06 and 0.2 in µg/mL.

3.7 Robustness

Deliberate changes in flow rate and organic phase in mobile phase ratio had little impact on retention time and % RSD is less than 2.0

(**Table 5)**.

Table 5: Results of robustness of (A) Captopril and (B) HCTZ

A

Parameter	Condition	Peak area ± SD, % RSD (n = 3)	USP tailing (n = 3)	USP Plate count (n = 3)
Flow rate (±10%)	0.22	2856051± 22941.18, 0.803	1.08	7747
	0.2	2974151± 8810.16, 0.296	1.12	7826
	0.18	3071727 ± 22357.66, 0.728	1.09	7936
Mobile Phase composition (± 10%)	66:34	2686226 ± 15636.84, 0.582	1.14	7721
	60:40	2966410 ± 4882.95, 0.165	1.16	7851
	54:46	3372226 ± 24223.56, 0.718	1.12	7938

B

Parameter	Condition	Peak area ± SD, % RSD (n = 3)	USP Tailing (n = 3)	USP Plate count (n = 3)
Flow rate (±10%)	0.22	2746251± 15639.48, 0.569	1.11	5023
	0.2	2945849± 6181.3, 0.21	1.15	5172
	0.18	3056289 ± 28895.54, 0.945	1.12	5206
Mobile Phase composition (± 10%)	66:34	2676572 ± 11497.37, 0.43	1.17	5085
	60:40	2944955 ± 4937.14, 0.168	1.14	5149
	54:46	3269340 ± 12546.85, 0.384	1.16	5263

mL/min-milliliter/ minute, ±-plus or minus, %RSD, SD-standard deviation -percentage statistical significance, n-number of tests, relative standard deviation,% v/v-percentage volume by volume

3.8 Forced Degradation studies of Teneligliptin and Remogliflozin

According to the ICH stability recommendations, studies of thermal degradation, basic degradation, acidic degradation, oxidative degradation, photolytic degradation, and reductive forced degradation were carried out. The investigations provided information on the circumstances in which the medication is unstable, and suitable safety measures were frequently taken during formulation in order to prevent any instability. The results of degradation are shown in **Table.6**.

Table.6: Forced Degradation results

Degradation condition	Captopril		HCTZ	
	% Assay	% Deg	% Assay	% Deg
Control degradation	100	0	100	0
Acid degradation	88.1	11.9	86.6	13.4
Alkali degradation	86.9	13.1	89	11
Oxidation degradation	83.7	16.3	85.7	14.3
Reduction degradation	89.5	10.5	90.8	9.2
Hydrolysis degradation	97.8	2.2	98.4	1.6
Thermal degradation	96.8	3.2	94.5	5.5
Photo degradation	97.6	2.4	95.8	4.2

IV.CONCLUSIONS

In this study, we develop a clear, focused, verified and clearly defined UPLC technique for quantitatively determining Captopril and HCTZ. Peaks were successfully resolved from one other and separate with adequate retention duration, showing the suggested approach to be quick, simple, practicable and economical under assay condition. Therefore the devised approach may be utilized for routine analysis of manufacturing samples and to check the quality of medication samples during stability studies.

V. ACKNOWLEDGMENT

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