International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 16, Issue 4, 2024

Original Article

A REVERSE PHASE ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY-PHOTO DIODE ARRAY ESTIMATION OF CAPTOPRIL AND HYDROCHLOROTHIAZIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Received: 14 Nov 2023, Revised and Accepted: 24 Apr 2024

ABSTRACT

Objective: This investigation demonstrates a stability-indicating and reliable "Reverse Phase Ultra-Performance Liquid Chromatography (RP-UPLC)" method to simultaneously quantify Hydrochlorothiazide (HCTZ) and Captopril in the pharmaceutical dosage form.

Methods: Hydrochlorothiazide and Captopril were separated by using C_{18} column (100 mm x 2.1 mm, 1.7µm) with an isocratic type of elution using mobile phase containing Acetonitrile+0.1% formic acid buffer (60:40), respectively with 0.2 ml/min flow rate. The wavelength used to detected at 210 nm to quantify Hydrochlorothiazide and Captopril.

Results: Captopril and Hydrochlorothiazide peaks were eluted with fine resolution at retention times 0.772 min and 1.679 min, respectively. In 5-30 µg/ml concentration ranges for each Captopril and Hydrochlorothiazide, the calibration graphs were linear, with regression coefficients of 0.9998 and 0.9995, respectively. The suggested Ultra-performance liquid chromatography approach has been shown as sensitive, precise, robust, accurate, specific and stability, indicating through the resolution of Captopril and Hydrochlorothiazide from its degradation-based compounds.

Conclusion: The established ultra-performance liquid chromatography technique was effectively extended to the evaluation of Captopril and Hydrochlorothiazide in the pharmaceutical dosage form, and the test results appeared satisfactory.

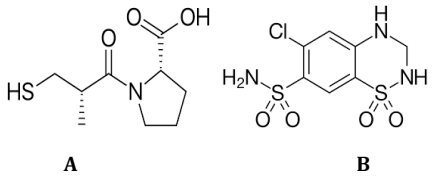
Keywords: Isocratic method, Development, Validation, RP-UPLC, Stability indicating

DOI: https://dx.doi.org/10.22159/ijap.2024v16i4.49857 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

The Angiotensin-Converting Enzyme (ACE) inhibitor [1] captopril is used to treat hypertension [2, 3] and some forms of congestive heart failure [4]. It goes by a number of trade names, including Capoten. When it came to treating hypertension, captopril was the first oral ACE inhibitor to be discovered. A lack of energy [5] is not a side effect, unlike beta-blockers [6, 7]. The medicine is often used with a diuretic because of the adverse pharmacological event of producing hyperkalemia [8, 9], which is common with most ACE Inhibitors. Captopril's primary actions include widening blood vessels and blocking the action of certain kidney enzymes [10]. When it comes to hypertension, cardiac issues such congestive heart failure and following myocardial infarction [11, 12], and diabetic nephropathy [13], these advantages stand out the most. On top of that, it has been seen to have a positive effect on mood in some individuals. This lines up with the finding that this drug exhibits potential antidepressant [14, 15] effect in animal screening models, despite the fact that one research has come out negative. There has been no mention of formal clinical studies including people with depression. Cancer therapy [16] using it has also been explored. It was also shown that some metallo-β-lactamases might be inhibited by captopril stereoisomers [17-19].

One common diuretic for hypertension and edema caused by fluid retention is hydrochlorothiazide. In addition to lowering the incidence of kidney stones in those with high urine calcium levels, it is used to treat diabetic insipidus and renal tubular acidosis [20, 21]. When compared to chlorthalidone [22], hydrochlorothiazide's efficacy in preventing cardiovascular events is lower [23, 24]. When taken orally, hydrochlorothiazide may enhance the efficacy of other blood pressure drugs when given together in a single dose. Negative effects on renal function, electrolyte imbalances [25, 26] (such as low blood potassium and, less often, low blood sodium), gout [27, 28], high blood sugar, dizziness upon standing, and high blood sugar are all possible adverse effects. It has been observed that individuals with sulfa medication allergies are more likely to have hydrochlorothiazide allergies, however, this link lacks strong evidence. You may use it when you're pregnant, but it shouldn't be your first choice for this condition. It lowers the kidneys' water-retention capacity and is hence a member of the thiazide drug class. At first, this lowers blood volume, which lowers cardiac output (the amount of blood that returns to the heart) [29]. In the long term, it may reduce peripheral vascular resistance. This paper proposes a novel sensitive stability-indicating RP-UPLC procedure for the assessment of Captopril and Hydrochlorothiazide combination. The aim of the study is to estimate the pharma ingredients Hydrochlorothiazide and Captopril by using RP-UPLC.



A **B**

Fig. 1: Structure of (A) Captopril and (B) Hydrochlorothiazide

MATERIALS AND METHODS

Chemicals

Acetonitrile, HPLC-grade methanol, water were purchased from Merck India Ltd, Mumbai, India. APIs of Captopril Hydrochlorothiazide standards were procured from Glenmark, Mumbai. Sample formulation (tablets) from Euphoria India Pharma with a label claim of 25 mg each of Captopril and Hydrochlorothiazide tablets was used.

The instrumentation

Waters Acquity UPLC [30, 31] with quaternary pump, PDA (Photo Diode Array) detector with Empower 2.0 software was employed.

Method optimization

To optimize the chromatographic conditions, different ratios of phosphate buffer and the acetonitrile in the mobile phase with

isocratic and gradient mode was tested. However the mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention times. Finally a mixture of acetonitrile and formic acid with isocractic elution was selected as mobile phase because it results in a greater response of active pharmacy ingredient. During the optimization of the method various stationary phases such as C_8, C_{18} and amino phenyl columns were tested. From these trials the peak shapes were relatively good with C_{18} column of 100 x 2.1 mm, 1.7 µ with a PDA detector. The mobile phase flow rate has been done at 210 nm in order to obtain enough sensitivity. By using above conditions, we get retention times of Captopril and Hydrochlorothiazide were about 0.772 min and 1.679 min with a tailing factor of 1.02 and 1.09. The number of theoretical plates for Captopril and Hydrochlorothiazide were 5647, 6587 which indicate the column's successful output the % RSD for six replicate injections was around 0.26% and 0.17%, the proposed approach suggests that it is extremely precise. According to ICH guidelines, the method established was validated.

Validation procedure

The analytical parameters such as system suitability, precision, specificity, accuracy, linearity, robustness, LOD (Limit of detection), LOQ (Limit of quantification), forced degradation and stability were validated according to ICH Q2 (R1) guidelines [32, 33]. (ICH–International Chiral on Harmonization).

Preparation of buffer

Take 1 ml of formic acid in 1 L of HPLC grade water and filter through 0.22 µ filter paper.

Chromatographic conditions

The UPLC analysis was performed on reverse phase UPLC system with isocratic elution mode using a mobile phase of Acetonitrile and 0.1% formic acid (60:40) and C_{18} (100x2.1 mm, 1.7 µ) column with a flow rate of 0.2 ml/min.

Diluent

Mobile phase was used as diluent.

Preparation of the standard solution

Standard Captopril and Hydrochlorothiazide solution containing 20 µg/ml and 20 µg/ml was prepared by dissolving 20 mg of Captopril and 20 mg of Hydrochlorothiazide in 100 ml of mobile phase solvent blend. Further dilute 5 ml to 50 ml with diluents.

Preparation of the sample solution

Sample solution containing 20 µg/ml each was prepared by dissolving 20 mg each equivalent weight of Captopril and Hydrochlorothiazide sample (label claim 25 mg of Captopril and 25 mg of Hydrochlorothiazide) in 100 ml of mobile phase solvent blend. Further, dilute 5 ml to 50 ml with diluents.

RESULTS

The main analytical challenge during the development of a new method was to separate active Pharma ingredients. In order to provide good performance, the chromatographic conditions were optimized. Hydrochlorothiazide belongs to a category of diuretics and Captopril belongs to a category of Angiotensin-converting enzyme (ACE) inhibitor and the stability indicating method for these drugs using UPLC has not been reported till date. So a new method has been developed and validated as per ICH Guideline.

In acquiescence with ICH recommendations, the validity parameters were established [34-36].

System suitability

In System suitability, injecting standard solution and reported USP (United States of Pharmacopeia) tailing and plate count values are tabulated in table 1 [37, 38].

Table 1: Results of system suitability

System suitability parameter	Acceptance criteria	Drug name	
		Captopril	Hydrochlorothiazide
USP Plate Count	Not Less Than 2000	5647	6587
USP Tailing	Not More Than 2.0	1.02	1.09
USP Resolution	Not Less Than 2.0	-	6.98
% RSD	Not More Than 2.0	0.26	0.17

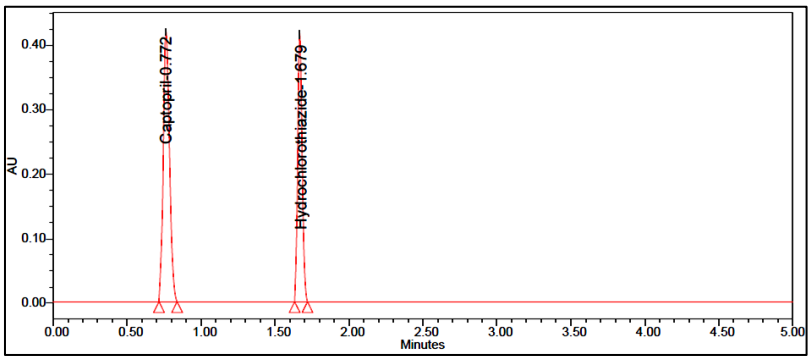


Fig. 2: Chromatogram of standard

Specificity

In this test method placebo, standard and sample solutions were analyzed individually to examine the interference [39]. The below fig. shows that the active ingredients were well separated from blank and their excipients and there was no interference of placebo with the principal peak. Hence the method is specific.

Linearity

During this work, the linearity of area response was checked for both Captopril and Hydrochlorothiazide. Chromatographed solutions with concentrations of 5-30 µg/ml for each of Captopril and Hydrochlorothiazide given linear peak response areas. The regression line equation, regression coefficient and Captopril and Hydrochlorothiazide calibration curves are shown in fig. 4.

Accuracy

The accuracy was determined by assay of Captopril and Hydrochlorothiazide in spiked Captopril and Hydrochlorothiazide samples according to proposed method. Three diverse quantities (50% quantity degree, 100% quantity degree and 150% quantity degree) [40, 41] of Captopril and Hydrochlorothiazide standards were put into samples. The results are given in table 3.

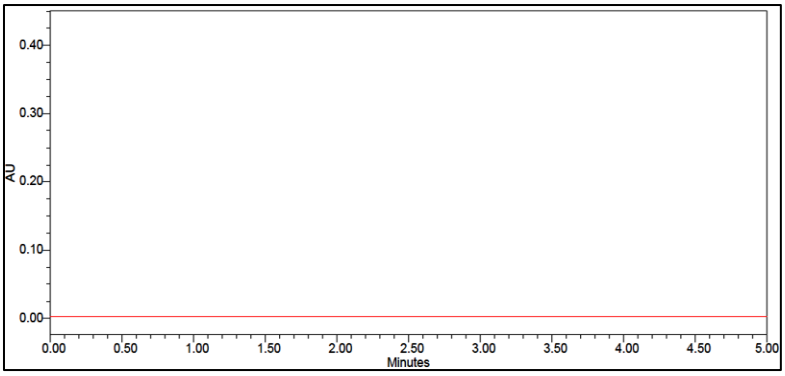
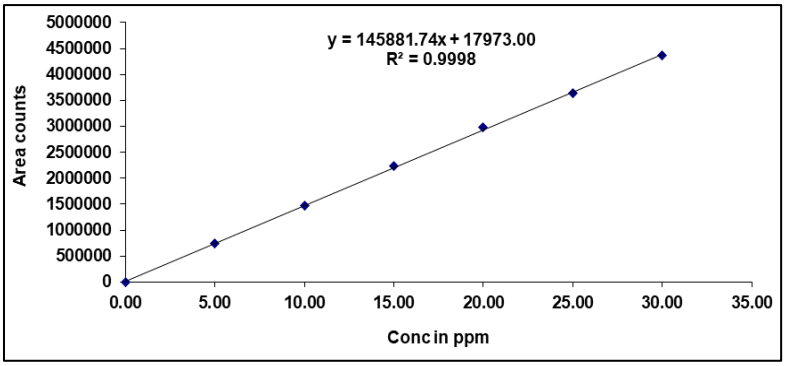


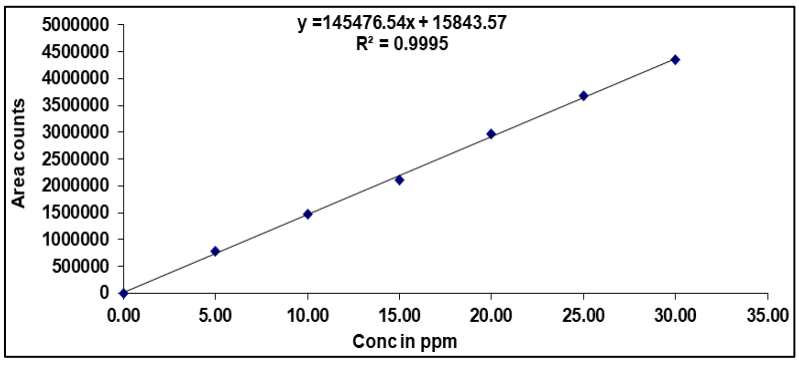
Fig. 3: Chromatogram of blank

Table 2: Linearity of captopril and hydrochlorothiazide

S. No.	Captopril		Hydrochlorothiazide	
	Conc. (µg/ml)	Area	Conc. (µg/ml)	Area
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



(A) Captopril



(B) Hydrochlorothiazide

Fig. 4: Calibration plots of (A) Captopril (B) Hydrochlorothiazide

Precision

The precision measurements were assessed using measurements of Captopril and Hydrochlorothiazide solution (20 µg/ml each) repeated six times within the day. The precision was validated by the RSD (Relative Standard Deviation) measurements of the Captopril and Hydrochlorothiazide peak areas, while the accuracy was validated by the Captopril and Hydrochlorothiazide percentage content assays. These results are given below table 4.

Intraday precision

Six replicates of a standard solution containing Captopril (20 µg/ml) and Hydrochlorothiazide (20µg/ml) were analysed on the same day

[42-44]. Peak areas were calculated, which were used to calculate mean, SD and %RSD values.

Intermediate precision

Six replicates of the standard solution were studied by various researchers, and on separate days different instruments were tested. The peak regions used to determine mean percent RSD values have been calculated. The results are given in the following table [45].

Inter-day precision

Six replicates of a sample solution containing Captopril (20µg/ml) and Hydrochlorothiazide (20µg/ml) were analysed on a different day. Peak areas were calculated which were used to calculate mean, SD and %RSD values. The present method was found to be precise as the RSD values were less than 2% and also the percentage assay values were close to be 100%. The results are given in table 5.

Table 3: Results of accuracy of (A) Captopril and (B) Hydrochlorothiazide

A

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

mean+SD (n=3), SD–Standard Deviation

B

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

mean+SD (n=3)

Table 4: Intraday precision results of captopril and hydrochlorothiazide

S. No.	Captopril			Hydrochlorothiazide		
	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
Mean		2982618	100.4		2956860	100.4
SD		13781.661	0.458		11815.147	0.420

mean+SD (n=6)

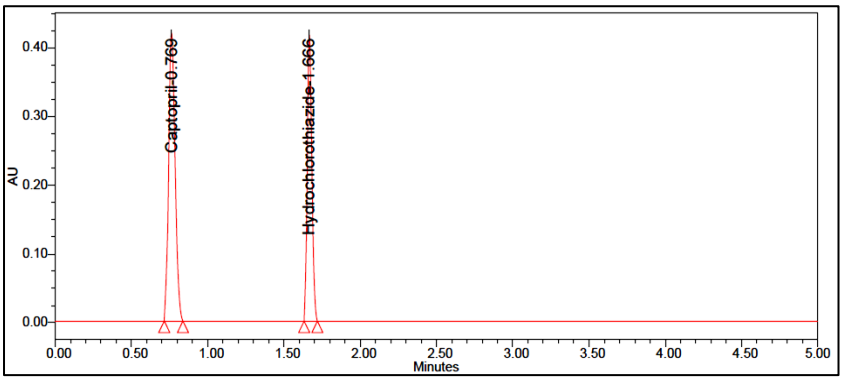


Fig. 5: Chromatogram of method precision

Table 5: Inter-day outcomes of accuracy of captopril and hydrochlorothiazide

S. No.	Captopril				Hydrochlorothiazide			
	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
Mean	2956035	99.5	2954046	99.4	2951862	100.2	2967423	100.8
SD	26226.348	0.888	25335.984	0.866	18303.228	0.622	16457.898	0.554

mean+SD (n=6)

LOD and LOQ

Both LOD and LOQ were measured utilizing a signal-to-noise methodology. LOQ and LOD were defined as the Captopril and Hydrochlorothiazide concentration levels that ensuing a peak height of 10 times and 3 times, respectively, the baseline noise.

Robustness

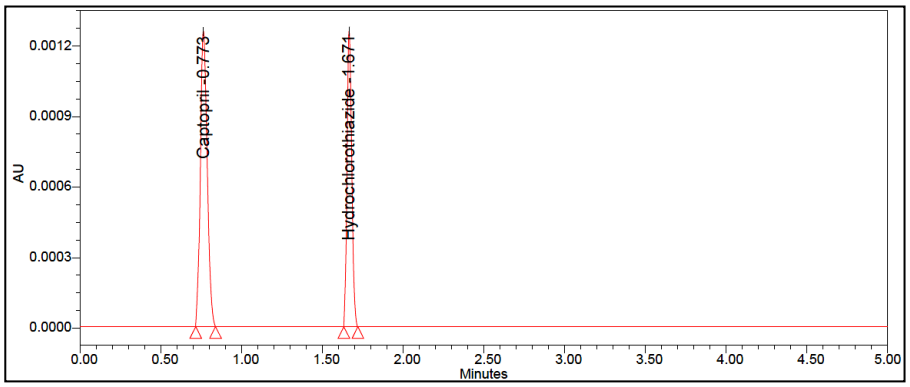
The robustness was measured using peak area measurements of Captopril and Hydrochlorothiazide solution (20µg/ml each) with considerably changed parameters in UPLC assay operating conditions. The changed parameters and peak areas obtained were presented in table 7 [46, 47].

Degradation studies

The Captopril and Hydrochlorothiazide sample was subjected into various forced degradation conditions to effect partial degradation of the drug. Studies of forced degradation have carried out to find out that the method is suitable for products of degradation [48, 49]. In addition, the studies provide details about the conditions during which the drug is unstable in order that measures are often taken during formulation to avoid potential instabilities [50].

Table 6: LOD and LOQ for captopril and hydrochlorothiazide

Captopril				Hydrochlorothiazide			
LOD		LOQ		LOD		LOQ	
Concentration	s/n	Concentration	s/n	Concentration	s/n	Concentration	s/n
0.06µg/ml	3	0.2µg/ml	10	0.06µg/ml	3	0.2µg/ml	10



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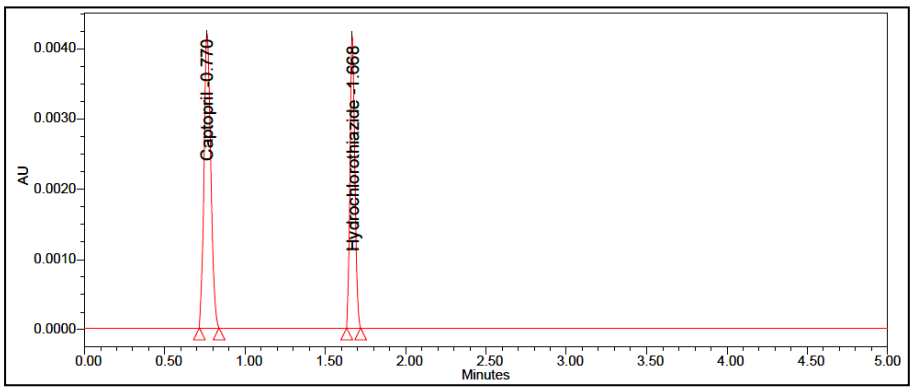

B

Fig. 6: Chromatogram of (A) LOD and (B) LOQ

Table 7: Robustness data of (A) Captopril and (B) Hydrochlorothiazide

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

mean±SD (n=3)

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

mean±SD (n=3)

Acid degradation

Acid degradation was done by using 1N HCl (Hydro Chloric acid) and 11.9% of Captopril and 13.4% of Hydrochlorothiazide degradation was observed.

Alkali degradation

Alkali degradation was done at 1N NaOH (Sodium Hydroxide) and 13.1% of Captopril and 11.0% of Hydrochlorothiazide degradation was observed.

Peroxide degradation

Peroxide degradation was performed with 30% hydrogen peroxide and 16.3% Captopril, 14.3% of Hydrochlorothiazide degradation was observed.

Reduction degradation

Reduction degradation was performed with 10% sodium bi sulphite solution, 10.5% Captopril and 9.2% Hydrochlorothiazide degradation was observed.

Table 9: Forced degradation results of captopril and hydrochlorothiazide

Degradation condition	Captopril		Hydrochlorothiazide	
	% 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

Thermal degradation

In thermal degradation, the sample was degraded to 3.2% of Captopril and 5.5% of Hydrochlorothiazide.

Photolytic degradation

In Photolytic degradation, the sample was degraded to 2.4% of Captopril and 4.2% of Hydrochlorothiazide.

Hydrolysis degradation

In hydrolysis degradation, the sample was degraded to 2.2% of Captopril and 1.6% of Hydrochlorothiazide.

All degradation results are tabulated in table 9.

DISCUSSION

A literature survey found that till today there were only two HPLC [51, 52] methods were reported. The simultaneous measurement of Captopril and Hydrochlorothiazide was not addressed by UPLC. We have developed a responsive, robust, and fast UPLC process to estimate Captopril and Hydrochlorothiazide. The UPLC method developed in our work is the first to concurrently measure Captopril and Hydrochlorothiazide in bulk and formulations of the tablet type. The ICH recommendations were considered taking into account while validating the established UPLC: Captopril and Hydrochlorothiazide analysis technique [53, 54]. Excipients in tablet formulation and mobile phase ingredients were not observed to interfere with Captopril and Hydrochlorothiazide elution. The selectivity was supported by the chromatograms (fig. 2, 3 and 5) of selectivity [55].

The main target of the chromatographic method is to get the separation of Captopril and HCTZ. Different trails using standard solution were made by using various mobile phases containing buffers like orthophosphoric acid, water with different pH values (2–4) and using organic modifiers like acetonitrile, methanol in the mobile phase and various stationary phases like C_8, C_{18}, and Phenyl. Based on these trails it was found that peak shape of Captopril and HCTZ were relatively good on C_{18} column in mobile phase formic acid buffer and acetonitrile. Using mobile phase A at different pH's, mobile phase B as acetonitrile, flow rate 0.2 ml/min injected standard solution on C_{18} 100 mm length, 2.1 mm ID column and 1.7 µm particle size using isocratic programme. From the trails, it was found that using formic acid as buffer, the separation is reasonably good, and hence, formic acid is selected as a buffer. The results clearly indicated that on a C_{18} column 100 mm length, 2.1 mm ID with 1.7 µm particle size and formic acid as buffer and acetonitrile as organic phase with an isocratic programme of 40:60 with a post-run

time of 5 min at a detection wavelength 210 nm was successful in the separation of Captopril and HCTZ. Under the above conditions, results were as follows: retention time of Captopril was 0.772 min, with a tailing factor of 1.02, number of theoretical plates (N) was 5647 and % RSD for six replicate injections was 0.26% and retention time of HCTZ was 1.679 min, with a tailing factor of 1.09, number of theoretical plates (N) was 6587 and % RSD for six replicate injections was 0.17%. For Captopril and Hydrochlorothiazide, the RSD percentage of peak area variability were 0.26% to 0.17%, demonstrating admissible system precision [56, 57]. The accelerated degradation tests demonstrate the Captopril and Hydrochlorothiazide's vulnerability to degradation in basic, heat, UV, acidic, as well as oxidative circumstances. Peak purity of stressed samples of Captopril and HCTZ was checked by using a photodiode array detector on Waters UPLC, and the purity angle is less than purity threshold in all the stress samples, demonstrating the homogeneity of analyte peak. In the present study, we intended to explore a specific, sensitive, and new HPLC method towards the analysis of Captopril, HCTZ.

CONCLUSION

The proposed research work is found to be promising and less time-consuming with the minimum amount of solvent utilization for method development. The developed method proved that the method is specific, accurate, precise, and robust for Captopril and Hydrochlorothiazide. Stress degradation studies revealed that Captopril and Hydrochlorothiazide withstand thermal, photo and hydrolysis conditions. At the same time, acidic, alkali, oxidative and reduction degradation also occurred for both drugs. The developed method and obtained statistical data manifested that designed protocol is simple, rapid and economical for the estimation of Captopril and HCTZ APIs (Active Pharmaceutical Ingredient) and pharmaceutical formulation.

ACKNOWLEDGEMENT

The authors were thankful to the management of P B Siddhartha college of Arts and Science for their encouragement.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

David Raju has collected the literature and information about the drug. Manoranjani has carried out the research samples and prepared the manuscript. Satyadev supported solution preparation in analysis and review the manuscript.

CONFLICT OF INTERESTS

Declared none

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